July 2021

EVALUATION OF COOL-SEASON ANNUALS EFFECT ON SOIL HEALTH IN WARM-SEASON PERENNIAL PASTURES IN SOUTHESTERN US.

Paola C. Muela Negrete

Louisiana State University and Agricultural and Mechanical College

EVALUATION OF COOL-SEASON ANNUALS EFFECT ON SOIL HEALTH IN WARM-SEASON PERENNIAL PASTURES IN SOUTHESTERN US.

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfilment of the
requirements for the degree of
Master of Science

in

The School of Plant, Environmental, and Soil Sciences

by
Paola Carolina Muela Negrete
B.S., Zamorano University, 2017
M.S., Louisiana State University, 2021
August 2021

Acknowledgments

First and foremost, all praise and thanks goes to Jesus Christ for his sweet love and words throughout my life and research work. It is because of him I am alive.

I cannot express enough thanks for the continued support and patience of my major advisor, Dr. Lisa Fultz. I am grateful for the learning opportunities, advice, and words of encouragement she has provided. I am grateful to have had the opportunity to be one of her graduate students and to learn from her excellent work ethic and passion for science. I truly admire her. I would also like to thank all the people involved in the NIFA project, including Dr. Montgomery, Dr. Kun Jun Han, Dr. Bisoondat Macoon, and Dr. William Pitman. As well as the committee members Dr. Tubana and Dr. Beasley.

I am extremely grateful for my parents' love, support, care, and prayers. Who I am as a professional and as a human I owe to them. Even when we are physically apart, their love is always with me and mine with them. I am thankful for my sister's love, support, and care during this process. Without her, my time as a graduate student at LSU would not have been the same. I am blessed to have her as sister and friend, she is my person.

Thanks to all my friends who are like family to me. My special thanks goes to Gabriel who is like a brother to me. He is always making me laugh and reminding me to enjoy life. I express my thanks to Juan, Hector, Ana, Paulina, Hannah Rose, Andrea Suazo, and Carolyn. They are a blessing in my life. Lastly, my sincere gratitude to goes to Emiliano Samaniego, my friend and psychologist who has helped me through difficult times.

Table of Contents

List of Tables

List of figures

Abstract

Perennial grass crops represent approximately 8 million hectares of the land area of the humid lower southeastern United States. These forage crops receive high rates of fertilizer, especially nitrogen (N), and near monoculture remains have often been treated with repeated applications of herbicides. Pasture management is crucial to improve soil properties in pasturelands. Common pasture management practices include introducing cool-season multispecies in warm-season pasture systems and forage harvest frequency of pasture systems. It is known that cool-season multispecies in warm-season pasture systems ensure cattle feeding during winter season and have beneficial effects on soil microbial biomass, soil organic matter (SOM), and enzymatic activity. Forage harvest frequency is needed in pasturelands to regenerate after intensive grazing, contributing to soil nutrient returns and reduce soil compaction. In this study three sites were evaluated, two in Louisiana and one in central Mississippi to determine whether soil health is enhanced by overseeding different species of cool-season annual treatments in two warm season pastures bermudagrass (*Cynodon dactylon* (L.) Pers.) and bahiagrass *(Paspalum notatum*). For this, the evaluation of microecological and chemical properties of soil were included to analyze the impact of pastureland management practices and the impact of species on soil health. Harvest frequency rates were including in plots to evaluate two harvest frequency rates at 4-week, at 8-week, and a cool-season annual mulch. Samples were collected at two depths, 0-10cm and 10-20cm to evaluate how soil properties fluctuate through soil profile. The methods used to analyze microecological properties incorporate fatty acid methyl ester (FAMEs), and enzyme assays (β-glucosaminidase and β-glucosidase). The chemical properties measured included pH, total carbo (TC), total nitrogen (TN), nutrient concentrations, SOM, and inorganic N. Significant changes over time were observed in several soil properties, one of these was SOM, which changed over

time in all sites. Depth significantly influenced majority of soil properties in this study, normally soil properties decreased when soil depth increased. Absolute abundance of fungi to bacteria ratio significantly increased under an 8-week harvest frequency treatment in 2020 in one site. Overseeding legumes, annual ryegrass, and grass increased potential β-glucosaminidase in soil in Louisiana. Relative abundance of arbuscular mycorrhiza fungi and Gram-negative bacteria increase in bottom depth of soil. General responses to harvest frequency treatments suggest that 8-week harvest frequency is more beneficial for most soil properties than 4-week harvest frequency.

Chapter 1. Introduction

1.1. Perennial grass pastures

Warm-season perennial grass pastures occupy 29% of land use of the United States, with about 106 million hectares of pastures and rangelands around the country (USDA, 2019). Perennial grass crops, predominately the warm-season grasses, bermudagrass (*Cynodon dactylon* (L.) Pers.) and bahiagrass *(Paspalum notatum*), represent approximately 8 million hectares of the land area of the humid lower southeastern United States, including states like Alabama, Arkansas, Louisiana, Florida, Mississippi, South Carolina, and eastern parts of Oklahoma and Texas. (Nickerson et al., 2011). In Louisiana, on the 684,907 hectares designated for pastures the main forage crop for permanent pastures is bermudagrass (60 %), followed by bahiagrass (40 %) (USDA, 2017). In 2015, Louisiana experienced an increase in cattle numbers for the beef cattle industry, meaning the area designated for pastures increased as well. On the other hand, Mississippi's pastureland represents around 1.4 million hectares, mostly for beef cattle (Byrd & Layton, 2018). Pasturelands in Mississippi contribute almost $400 million in sales with beef cattle and forage production by year (Meter & Goldenberg, 2014). Bahiagrass is grown on more than 404,686 hectares in Mississippi, principally utilized for grazing and hay. Bermudagrass represent most of the Mississippi pasturelands with 1.1 million hectares (Lemus, 2018).

Bermudagrass is a warm-season perennial grass species normally found under tropical and subtropical climates. This grass was introduced to the United States around the 18th century in Georgia and taken to other states (Twidwell, 2000). Because this grass will remain dormant under cool temperatures, it is normally utilized by farmers in regions with high temperatures and mild winters. Although bermudagrass is used around all United States, it is very popular in the southeastern United States during the warm season for pasture and hay production (Silveira et al., 2007). In Louisiana, common bermudagrass is used as a pasture forage. The advantages of this perennial grass as a pasture forage include its production of deep roots and vigorous growth that is well adapted to Louisiana soils and weather conditions. Nitrogen (N) applications, known to improve inorganic nutrient absorption, directly impacts yield and quality of bermudagrass (Haby et al., 2008). However, bermudagrass is not a high protein forage which means it is necessary to include supplements for complete cattle nutrition (Han et al., 2012). Furthermore, this pasture has a high N fertilizer need between 84-280 kg/ha, and for this reason it can be very expensive to manage extensive bermudagrass areas. Additionally, N fertilizer rates in excess of what bermudagrass can efficiently use may lead to pollution in water resources affecting aquatic environments. For this reason, looking into sustainable options is essential to assure productivity in lands with bermudagrass without affecting environmental ecosystems.

Bahiagrass is grown broadly in the southeastern United States because it is a long-lived, perennial warm season grass. It has a variety of uses including serving as a pasture forage, as well as hay production, erosion control, and wildlife habitat. The advantage of planting this grass includes the ability of bahiagrass to grow on poorly drained soils, to tolerate shade more than bermudagrass, and its potential use in silvopasture practices (Hancock, 2006). Bahiagrass tolerates low soil fertility (except N), low soil pH, and is best adapted to sandy soils. Bahiagrass also responds to high N fertilizer rates (112-224 kg/ha) although grazing quality is reduced during mid-summer (Hancock et al., 2010). Additionally, bahiagrass biomass is mostly concentrated at the soil surface, providing considerable storage of organic and inorganic nutrients. This characteristic of bahiagrass makes it very competitive and reduces risks of weed invasion. However, for forage production bahiagrass is not well suited to fulfill high energy livestock demands requiring either the introduction of high protein crops or inclusion supplements into the cattle's diet (Gates, Quarin, & Pedreira, 2004).

1.1.1. Pasturelands managements

Pasture management practices can be highly valuable because they positively influence soil health which is related with pasture sustainability (Alemu et al., 2019). Inadequate management in pasturelands can lead to soil degradation and poor water quality (Amorim et al., 2020). Several factors affect forage species yield including grazing management and weather conditions. Grazing management is essential for economic returns, for forage quality, and for maintaining benefits of pastures and cool-season annuals (Donaghy, 2009). Harvest frequency is an important part of grazing management which can be determined by understanding the physiology of the pasture and cool-season annuals. Harvesting at a shorter frequency interval leads to decreased pasture persistence, reduced root elongation, decreased dry matter yield, and lower production (Tulley, 2015). Studies reported most noticeable improvement in soil health when harvest frequency interval increased, reporting better results in an 8-week harvest treatment (Simard et al., 2020). Weather condition is a factor that unlike grazing management cannot be anthropologically controlled. Extreme rainfall events affect principally pasture growth, contribute to soil nutrients run-off, and reduce pasturelands productivity (Derner et al., 2008). However, for purposes of this study harvest frequency will be assessed as a principal factor influencing pasturelands soil health.

1.2. Cool-season annuals

Introducing cool-season annuals, such as brassicas, grasses, and legumes, into permanent pastures may increase productivity and enhance soil health in grazing systems (Sanderson et al., 2004). For southeastern United States areas, the establishment of cool-season annuals as cover crops into warm-season perennial pastures helps to decrease the forage deficit periods providing grazing material in the winter and spring that reduces the need for supplemental feed (Leanne et al., 2018). Furthermore, some cool-season annuals have a higher nutritive value than most warm-season grass pastures and provide weed control during the spring season (Evers, 2011). Cool-season annual grasses are also commonly known for their tolerance to continuous grazing pressure (Brummer & Moore, 2000). Additionally, cool-season annual legumes are commonly used in areas with mild winters. Legumes are used less often for overseeding warm-season perennial pastures than grasses and brassicas, even though legumes are known for their high protein content as forage crops and for improving soil fertility by fixing atmospheric N (Iglesias & Lloveras, 1998). Planting brassicas resulted in increased soil aeration and enhanced availability of water and nutrients in the soil (Chen et al., 2014). These groups of cool-season annual species have their own advantages and limitations under warm-season perennial pastures and mixing them together may be a good way to use their benefits for a healthier and productive pastureland.

1.2.1. Grasses

Primary cool-season annual grasses used for overseeding warm-season perennial grasses are annual ryegrass (*Lolium multiflorum* L.), wheat (*Triticum aestivum*), and oat (*Avena sativa* L.). These cool-season annual grasses can tolerate cool temperatures and are productive in shorter photoperiods and higher soil moisture (Cool-Season or Warm-Season Grasses, 2018). Wheat is the most cold-tolerant of these grasses, however, its yield is typically lower than the other two grasses. Annual ryegrass, well adapted to most soil types, particularly poorly drained soils, is the most popular cool-season annual for overseeding in the southeastern United States (Evers, 2005). Along with this, cool-season grasses are beneficial for soil health because of the organic matter contributions they provide, as well as stimulating microbial communities (Wick et al., 2017).

The environmental services provided by grasses include nutrient scavenging and control of soil erosion, and because of this, water quality is conserved and improved (Wick et al., 2017; Stock et al, 2004). The addition of cool-season grasses into grazing systems can enhance soil physical properties, especially in the superficial layer of these soils. Annual ryegrass and wheat can increase aggregate formation because of their rooting depth which can also help to reduce soil compaction (Liu et al., 2005; Villamil et al., 2006). Therefore, improvements in aeration and water retention can result from the improved root penetration these grasses provide (Wick et al., 2017). Cool-season grasses also can retain excess nutrients remaining in the soil, particularly N, and make it available for the next crop production cycle. Phosphorus (P) uptake and incorporation into aboveground biomass is another attribute of these grasses (Liu et al., 2015). Wheat roots, in particular, can reach deep into the soil profile especially during the winter season which contributes to reduced erosion, scavenged nutrients, and increased moisture holding capacity (Thorup-Kritensen et al., 2009).

Some grasses like oats (*Avena sativa L.*) increase arbuscular mycorrhizal fungi (AMF) populations, which improves soil fertility and forage quality (Lehman et al., 2012). Arbuscular mycorrhizal fungi are important microbial communities because of their ability to tolerate rough environmental conditions and their contribution to plant P uptake. The utilization of cool season grasses also can increase soil organic matter (SOM) and total carbon (TC) in soil because of the reduction in soil disturbance and the addition of more organic residues into the soil (Liu et al., 2005).

1.2.2. Legumes

Cool-season legumes are a major tool to enhance soil health. Their contribution of N through N_2 fixation and high-quality forage production are two major benefits of utilizing legumes as a forage

(Nelson and Burns, 2006). Consequently, the scavenging of N reduces nitrate leaching and the increase of soil N through legumes can reduce supplemental N needs (Wick et al., 2017), which may lower the use of high-cost fertilizers in perennial pastures. Also, benefits in soil physical properties are documented, these included improved soil aggregation and stability of soil aggregates (N'Dayegamiye et al., 2015). Aggregate formation is associated with increased microbial activity and increased organic material in the root zone (Jastrow & Miller, 1998). According to N'Dayegamiye et al. (2015), planting crimson clover (*Trifolium incarnatum*) resulted in the highest increase of soil aggregate proportion compared to the control in a corn (*Zea mays*) field. Furthermore, legumes can reduce soil bulk density in the topsoil layer (0-10 cm) and increase total soil porosity. These physical properties are related with increased SOM and increased root system surface area (Villamil et al., 2006).

One of the benefits of legumes specifically related to soil chemical properties includes improved N cycling. Legumes tend to scavenge available N in the soil first and then form symbiotic partnerships with bacterial communities which fix atmospheric N (N_2), a portion of which is mineralized and readily available for the next crop (Wick et al., 2017). Jani et al. (2015) reported that crimson clover released 50% of the N in the root biomass (from fine roots mainly) within one week and N in coarse roots was released within eight weeks, increasing inorganic N in the soil. The potential of white clover (*Trifolium repens L.*) in terms of N-fixation rates range between 600-700 kg N $ha^{-1}year^{-1}$ in a non-grazed system. However, in a well-managed grazed pasture the N-fixations rates dropped to around 380 kg N $ha^{-1}year^{-1}$ (Caradus et al., 1996).

Legumes tend to benefit soil biological properties as well. There are studies reporting greater fungal and bacterial populations, greater biomass, and higher enzyme activities when introducing legumes to organic farming systems (Biederbeck, Zentner, & Campbell, 2005). Crimson clover increased β-glucosidase and β-glucosaminidase (NAGase) activities, contributing to C and N cycling in soils on a conventional to organic transition system (Liang, Grossman, & Shi, 2014). Furthermore, studies had reported that red clover (*T.pratense L.*) can increase soil organic matter and soil microbial activity when used as cover crop in conventional management systems (Mckenna et al., 2018).

1.2.3. Brassicas

Brassica's species provide different root characteristics, leaf architecture, and plant growth rates to suppress weed invasion (Sanderson et al., 2005). Brassica species can improve aeration, enhance drainage, and increase nutrient uptake (Williams & Weil, 2004). Most common species of brassicas used as cover crops included rapeseed (*Brassica napus* L.), Daikon radish (*Raphanus sativus* var. Longipinnatus), and turnip (*Brassica rapa* subsp. rapa) (Wick et al., 2017).

Brassicas can quickly grow tap roots with broad rooting depths (Weil et al., 2009). Radish and turnip have tap roots that grow long and wide into the soil, improving soil structure (Williams & Weil, 2004). Moreover, this allows root penetration in compacted soils, retrieving access to soil moisture and nutrients (Chen & Weil, 2010). Nutrient uptake and release, especially for N, is part of brassicas' benefits on soil chemical properties. According to Lounsbury and Weil (2020), the early season planting of spinach (*Spinacia oleracea*) into forage radish residues increase nitrate levels, which boost yields for this crop. Also, cool-season brassicas can increase P availability in soils (Wick et al., 2017). Brassicas are known for their potential as bio-fumigators that activate bacterial and fungal communities suppressing weeds (Haramoto & Gallandt, 2004; Wick et al., 2017).

1.2.4. Cool-season annuals mixtures

Utilizing complex mixtures of various cover crop species in dormant sod of widespread warm-season perennial grass crops of the region has potential to transform forage crop production from chemical input-based systems to ecologically driven production. Additionally, including cool-season annual mixtures may reduce fertilizer requirements through improved soil fertility and soil health (Rutkowska et al., 2014). Cool-season annuals also reduce competition from weeds through complementary crop resource use and enhance sustainability of both the areas of forage crop production and the associated environment (Han et al., 2013; Dabney, 2001; Lithourgidis et al., 2006). An individual clover species will not consistently be the superior specie under the variable environmental conditions of the lower south-central states. As illustrated by differences in primary growth period between legume species as illustrated by crimson clover, and white clover, and differing effects of seasonal weather patterns, and other environmental conditions on growth responses among adapted clover species (Han et al., 2012). Thus, legume mixtures can enhance the probability of successful legume plantings. Mixtures of cool-season annuals (legumes, brassicas, and grasses) reduce soil erosion and reduce nitrate leaching while contributing organic matter to the soil. Proper management of these cool-season annual combinations can increase soil porosity, improve soil structure, and stimulate microbial activity, enhancing soil health in row crops and grazing lands (Gollner et al., 2020).

Kramberger et al., (2014) reported that utilizing mixtures of crimson clover and annual ryegrass provided enough N to fully grow corn in arid regions. Other studies reported that cool-season annual grass mixtures interseeded into bermudagrass sod, produced high possible animal performance, increased breeding worth (BW) gain/hectare, and profitability (Beck et al., 2006). Combinations of brassicas and grasses reported increases in aggregation and rooting, producing increases in total porosity in surface soils (Villamil et al., 2006; Wick et al., 2017). Ultimately, multi-species mixtures can offer greater benefits to soil and ecosystem health in grazing systems, facilitating forage quality and quantity to optimize cattle performance (Kelly et al., 2021).

1.3. Soil health

Soil health is defined as the soil's aptitude to preserves productivity, accompanied with diverse soil organisms and environmental functions provided by it to have healthy plants, animals, and people (Cano et al., 2018). Soil health can be negatively affected by several weather conditions or poor management implemented as part of agricultural practices. Soil health is related to carbon sequestration, water quality, and biodiversity (Lichtfouse et al., 2009). Healthy soils are a result of good soil management like diversification of plant species using cover crops, polyculture, and crop rotation (Dubey et al., 2019). Indicators of soil health can be physical, chemical, or biological. Physical properties of soil health are associated with texture, aeration, structure, porosity, and water movement and retention. Chemical properties that indicate soil health can be assembled into three groups of parameters, such as soil acidity, soil carbon status, and nutrient availability (Schoenholtz et al., 2000). Biological properties as indicators of soil health are the components that show the beneficial functions of ecosystems in the soil. Some examples of biological properties are the soil organic carbon (SOC), soil microbial community composition, and enzymes catalyzing biochemical reactions (Bhowmik et al., 2019).

Soil health indicators help to increase the value of pastures. Healthy soils assure better quality forage and resilience of the soils to resist weed infestation and have less disease retention (USDA, 2016). Therefore, the inputs of chemical herbicides would be reduced. This may also be applied to fertilizer requirements which may be reduced if nutrient cycling and availability is adequate to meet plant needs, which translates into decreased costs of production. Examples of management practices for healthy soils include minimum tillage, cover crops, nutrient management, and residue mulch. The implementation of practices to improve soil functions through the maintenance or enhancement of soil physical, biological, and chemical is needed aides in the management of soil health. Soil physical, biological, and chemical properties are not mutually exclusive, interacting in complex ways to maintains soil functions like nutrient cycling, erosion mitigation, and improved soil moisture.

Soil physical properties should be considered and carefully managed (when possible), especially the most important soil physical properties which include soil texture, soil structure, and soil porosity. Soil texture is a highly variable property and is one that cannot easily be changed. Soil texture has the potential to impact a variety of soil functions, including water infiltration and retention and soil fertility (Idowu et al., 2019). Management decisions that take soil texture into account can promote sustainable productivity. Soil structure in the other hand, directly affects plant growth. A friable soil does not necessarily mean a good soil, even though roots can grow rapidly in it, their nutrient and water uptake may be reduced due to the limited contact with solid and liquid phases in soil. However, the presence of macropores in soil is essential because they benefit the extent of the root system. Also, these micropores contribute to increased microorganism populations, providing niches for them to occupy (Passioura, 1990).

Chemical properties typically focus on soil nutrient content and cycling in addition to soil pH. In general plants have basic nutritional requirements to progress through and complete their reproductive stages. When soil nutrient levels are reduced this results in nutrient deficiencies, restricting plant growth (Reeve et al., 2016). Alternatively, excess nutrients, like N, can also result in problems with plant growth, like excess growth resulting in lodging (Griffin, 2008). Nitrogen is an essential nutrient, the supply of which can be affected by several factors including pH, electrical conductivity (EC), cation exchange capacity (CEC), and SOM. Also, many soil microorganisms are affected by the pH of soil and their optimal function is achieved in a pH range from 6 to 7 (St. Luce, et al., 2011).

Soil biology, which compromises both living and dead organic material, has long been of interest, although management recommendations continue to lag behind those associated with physical and chemical properties. (Pankhurst et al., 1995). However, biological properties have begun to receive much more attention as they are the key drivers to improving many soil functions including nutrient cycling, SOM retention, and aggregate stability (Scheu, 2002). Specifically, soil microorganisms are responsible for nutrient transformations in soils and contribute to soil fertility and soil structure (Lee & Pankhurst, 1992). Changes in soil biological properties can lead to notable changes in soil physical and chemical properties as well (Turco et al., 1994).

1.4. Soil microbiology

The soil is an important source of nutrients; it provides structural support and is a reservoir for agricultural crops and plants (Voroney & Heck, 2015). Soil harbors biological organisms and many microbial entities the principal functions of which include nutrient recycling, sequestration, and supply to plants (Purakayastha et al., 2019). Soil microbial communities are sustained across an extensive group of physical and chemical conditions. Soil pH, salinity, CEC, and aggregate structure are a few examples of soil properties correlated with soil microbiology. Microbes produce carbon dioxide (CO_2), organic acids, and hydrogen ions that affect soil pH (Tate, 2000). Also, the variation in production of root exudates can result in variations of microbial and enzyme activities (Reeve et al., 2015).

Fungi is a particular interest for its ability to decompose dead or living organic matter, move rapidly through soil surface, and their role in soil aggregate formation (Knudsen, 2006). Many fungal organisms can fix CO_2 with the help of enzymes like pyruvate carboxylase (Paul, 2014). Several studies have also demonstrated the importance of AMF in soil health. Arbuscular mycorrhizal fungi are known for enhancing water efficiency and nutrient availability to plants, contributing to soil nutrient cycling, and improved salinity tolerance and drought tolerance of plants (Selvasekaran & Chidambaram, 2020). Fungi commonly create associations with other organisms and form symbiotic relationships (Went & Stark, 1968).

Bacteria play an important role in soil, contributing to the decomposition of organic material from enzymes in soil (Johns, 2017; Paul, 2014). They are decomposers, consuming simple sugars and carbon compounds, including plant residues and root exudates. Also, bacteria can have a complicated relation with plants by either forming mutualist relationships, providing access to nutrients, or acting as pathogens. The parasitic bacteria acquire their nutrition from a host, by attacking and ultimately harming the host (Johns, 2017). Additionally, some bacteria can obtain energy apart from carbon compounds in soil, utilizing N, sulfur, hydrogen, and iron instead (Hoorman, 2011). These adaptations to a variety of conditions results in bacterial typically contributing the most to microbial population numbers.

Enzyme activity is an important part of soil microbiology and is considered an indicator of soil health. The most common enzymatic activity in soils involves cytoplasmic activities associated with intermediary metabolism, like producing energy (Kohler et al., 2020). Extracellular enzymes play a very important role in terms of ecosystem function. Soil enzyme activity may be affected by environmental conditions like temperature changes, different topography, or soil type, and by anthropogenic activities (Gianfreda & Ruggiero, 2006). β-glucosidase is an enzyme of interest for soil microbiology as it is commonly associated with the C cycle and SOM (Almeida et al., 2015). β-glucosidase produces energy for soil microorganisms through the hydrolysis processes. β-glucosidase enzyme activity is affected by abiotic and biotic conditions and in monoculture systems this enzyme tends to lower its activity (Alkorta et al., 2003). β-glucosaminidase or NAGase, is involved in the degradation of chitin, a biopolymer in the soil which offers an important pool of organic C and N. NAGase activity has also been highly related to N mineralization in soil (Ekenler & Tabatabai, 2003).

1.5. Rationale for research

Soil health is an important factor for land managers and farmers. If soil health is negatively affected by erosion, poor water retention, reduced SOM, and lack of nutrient availability; pastures, forage yield, and livestock performance will struggle as well. Without proper soil health management, forage and cattle productivity cannot be improved, causing greater costs for producers generated by using supplemental protein to meet feeding requirements of the cattle. Additionally, excessive applications of nutrient fertilizers have negative environmental effects, including degrading aquatic ecosystems by nutrient-rich runoff. Negative environmental changes involve less predictable rainfall patterns in Louisiana and Mississippi, which normally used to be uniform through the summer season and directly affects grazing lands production. Enhanced soil health may increase the resilience of pasturelands to changes in rainfall frequency and fluctuations in weather conditions. Therefore, forage crop diversity will increase distributing forage production over a longer period and consequently susceptibility to droughts will reduce. Economics, environmental concerns, and less-predictable weather patterns are challenges that highlight the need for enhanced options for perennial forage crops in the region. The lack of information regarding benefits on soil health of overseeding cool-season annuals into warm-season perennial pastures, leads to a list of unanswered questions in the search for best management practices the southeastern United States. Ultimately, the goal of this study was to evaluate the effect of overseeding with diverse cool-season annuals on soil health in bermudagrass and bahiagrass perennial pastures in two states in the southeastern United States. Additionally, evaluate the effect of harvest frequency treatments and mulching on soil health in bermudagrass and bahiagrass in the southeastern United States. This includes the assessment of soil physical, chemical, and biological properties in three different locations in Louisiana and Mississippi.

Chapter 2. Materials and Methods

2.1. Site descriptions

For this study, three sites were selected to replicate the experiment in a develop a warm-season perennial pasture system, which could be bahiagrass or bermudagrass. The warm-season perennial pasture was overseeded with multiple species of cool-season annuals using three functional groups (brassicas, grasses, and legumes). This study took place from 2017 to 2020 at each site, however soil sampling was done in 2017, 2018 (sites only), and 2020. These sites were overseeded with combinations of cool-season annual treatments and three harvest-frequency treatments in a randomized complete block-design with three replications. The main plot treatment was harvest frequency with biomass collected at intervals of either 4- or 8-weeks, and an end of cool-season annuals mulch and was randomly selected. The subplot factor was the cool-season annual treatments allocated randomly within each main plot. The cool-season annual species utilized for the three sites were consistent and included Eco Till radish (*Raphanus raphanistrum* subsp. *Sativus L*.), Barsica forage rape, and Barkant turnip for brassicas. The grasses were Wrens Abruzzi cereal rye (*Secale cereale L*.), EK 102 wheat, Marshall annual ryegrass, and TAMO 606 oat. Legumes used included Durana white clover, AU Red Ace red clover, AU Don ball clover (*T. nigrescens L*.), and Dixie crimson clover.

The trial establishments were consistent between sites although randomization of treatments was unique to each location. The 9 subplots included 1) mixture of 10 cool-season annual species, 2) legume functional group, 3) brassicas functional group, 4) grasses functional group, 5) mixture of grasses and legumes, 6) mixture of grasses and brassicas, 7) mixture of legumes and brassicas, 8) annual ryegrass monoculture, 9) check which was the bermudagrass or bahiagrass not overseeded with cool-season annuals. All the cool-season annuals were planted in fall of each year from 2017 to 2020 for the three sites. Plot size was 2 m x 5 m with a 0.5 m-alley between each plot with cool-season annuals planted in a 1.5 m x 5 m strip down the middle of the plot.

Planting rates for cool-season annuals were consistent at the Hill Farm and Ben Hur sites. The Brown Loam site utilized different seeding rates. At Hill Farm and Ben Hur, the cool-season annuals were broadcasted seeded except for small grains & radish that were drill planted. The seeding rates at Hill Farm and Brown Loam were: annual ryegrass at 33.6 kg ha^{-1} and 22.4 kg ha^{-1} (monoculture and mixture), for cereal rye 56.0 kg ha^{-1}, oats at 67.3 kg ha^{-1}, for wheat at 67.3 kg ha^{-1}, for white clover at 5.6 kg ha^{-1}, red clover at 9.0 kg ha^{-1}, crimson clover at 13.5 kg ha^{-1}, ball clover at 5.6 kg ha^{-1}, turnip at 3.3 kg ha^{-1}, brassica forage rape at 4.5 kg ha^{-1}, and radish at 6.7 kg ha^{-1}.

2.1.1. Ben Hur

The Ben Hur site was established at the Louisiana State University Agricultural Center Ben Hur Farm in Baton Rouge, LA (30° 22' N, 91° 10' W). The annual average maximum temperature of this location was 18.6°C and the annual average minimum temperature was 7°C. The average annual precipitation was 1,540.5 mm. Between October 2017 to September 2020 the highest monthly accumulated precipitation occurred in April 2019 with 251.5 mm and the lowest precipitation was in March 2019 with 29.7 mm (Table 2.1). The highest average monthly air temperature was recorded in August 2019 at 29°C and the lowest was recorded in January 2018 with 8°C (Table 2.1). The soil type of this location is principally a Thibaut Series which is a very deep, poorly drained soil that is formed in clayey alluvium on fine-silty alluvium. These soils are frequently found on alluvial flats and on the alluvial plain of the Mississippi River. The slope for this soil is 0 to 1% and the depth to water table is about 46 cm to 91 cm (Natural Resources Conservation Service, 2019).

Table 2.1. Weather description from 2017-2020 for Ben Hur, Baton Rouge, LA.

	Monthly accumulate precipitation. (mm)				Average monthly air temperature (°C)		
Month	2017-2018	2018-2019	2019-2020		2017-2018	2018-2019	2019-2020
Oct.	91.9	99.8	198.4		21.4	22.7	21.8
Nov.	6.8.6	143.8	43.7		16.8	13.8	13.9
Dec.	151.1	235.0	62.0		11.3	11.9	13.2
Jan.	182.1	88.4	129.8		8.0	10.7	13.4
Feb.	102.1	66.0	157.7		18.4	17.1	13.9
Mar.	129.0	29.7	55.6		17.3	16.0	20.6
Apr.	138.4	251.5	183.9		17.8	19.7	20.2
May	88.9	246.4	124.5		26.1	25.4	23.1
Jun.	161.5	188.0	222.0		28.2	27.6	26.6
Jul.	107.7	182.1	226.3		28.8	28.2	27.6
Aug.	176.8	197.9	77.2		28.1	29.0	27.9
Sep.	85.9	64.5	58.2		27.4	28.3	25.2
Total	1484	1793.1	1539.3	Mean	20.8	20.9	20.6

Source: (United States Climate Data, 2021) and (Weather Underground, 2021).

For planting cool-season annual species (see Section 2.1 for rates) a cone drill was utilized with disk and broadcast. The cool-season annuals were not fertilized and the warm season pasture which was bermudagrass was fertilized by applying 33.6 kg N ha^{-1} as urea at initiation and after each harvest of each year from 2018-2020. Phosphorus and potash (0-0-60) fertilizer was applied in June of each year.

2.1.2. Brown Loam, MS

Site description

This Brown Loam was conducted at the Brown Loam Branch Experiment Station at Raymond, Mississippi (32°12’ N, 90°30’ W) beginning in 2017 and ending in 2020. The soil type in this site mainly was a Loring silt loam (2 to 5% slopes, moderately eroded). Loring soils typically have a depth to restrictive feature around 69 cm to 83 cm. Loring soils are a moderately well drained soil with a medium runoff class, their depth to water table is about 61 cm to 71 cm. This soil type is not frequently flooded or ponded and has a low available water capacity.

Table 2.2. Weather description from 2017-2020 for Brown Loam Station, Raymond, MS.

	Monthly accumulate precipitation. (mm)				Average monthly air temperature (°C)		
Month	2017-2018	2018-2019	2019-2020		2017-2018	2018-2019	2019-2020
Oct.	73.9	34.5	332.7		19.9	20.2	19.4
Nov.	18.5	196.6	40.6		15.3	10.9	11.1
Dec.	92.7	218.7	100.6		9.3	9.8	10.8
Jan.	102.4	205.7	343.7		5.5	8.4	10.5
Feb.	259.6	84.8	247.9		14.6	13.2	11.4
Mar.	162.3	101.9	133.9		15	13.1	19.2
Apr.	148.8	190.8	247.4		15.6	17.4	17.8
May	96.8	175.3	55.1		25	24	21.9
Jun.	103.6	134.1	136.7		27.6	25.7	26.1
Jul.	95	149.1	88.6		28.3	27.3	28.1
Aug.	103.9	126	226.6		27.3	28	27.0
Sep.	167.4	0.5	122.4		26.7	28	24.8
Total	1424.9	1618	2076.2	Mean	19.2	18.8	19.0

Source: Delta Agricultural Weather Center. Retrieved from: http://deltaweather.extension.msstate.edu/brown-loam-exp-stn

The subplots were separated by a 0.5 m alley and had an area of 1.5 m x 5 m and planted into an existing bermudagrass pasture. There was a 2 m alley separating main plots within blocks and a 3-m alley separating blocks. Although all nine cool-season annual treatments were seeded, only five overseeding treatments and the check were considered for this manuscript due to poor performance in the two-species mixture treatments. The five overseeded treatment groups considered were 1) 10-species mixture 2) grass functional group, 3) legume functional group, 4) brassica functional group, 5) monoculture annual ryegrass, and a check with the warm-season perennial grass sod not overseeded and no weed control.

For overseeding the cool-season annuals the equipment utilized was a Hege plot seeder (Kincaid Equipment Manufacturing, Haven, KS). They were no-till seeded in seven rows with a 20 cm separation during mid-October of each year (2017-2020). The seeding rates for the cool-season annuals were turnip at 3.4 kg ha^{-1}, rape at 4.5 kg ha^{-1}, radish at 6.8 kg ha^{-1}, wheat and oat at 67 kg ha^{-1}, annual ryegrass at 22 kg ha^{-1}, red clover 9 kg ha^{-1}, crimson clover at 13.4 kg ha^{-1}, white clover at 3.4 kg ha^{-1}, and ball clover at 3.4 kg ha^{-1}. These seeding rates were the same during the three-year study period. No fertilizer and herbicides were applied to the cool-season annuals, however during each summer fertilizer was applied for the bermudagrass growing season except in 2017. The fertilizer was applied in July and then in August starting in 2018 until 2020. The fertilizer rate was 316 kg ha^{-1} of 19-19-19 (N-P-K) in July and 60 kg N ha^{-1} as urea in August.

2.1.3. Hill Farm

The Hill Farm site was located at the Hill Farm Research Station in Claiborne Parish in north Louisiana. The station is part of the Louisiana State University Agricultural Center. The area utilized for this study was a bahiagrass pasture established before the beginning of this study in 2017. The elevation of the site was approximately 61 to 137m, with a mean annual precipitation of 1,194 to 1,626 mm, and a mean annual temperature around 11°C and 24°C, and a frost-free period of approximately 200 to 259 days. The main soil type in the area was a Darley gravelly loamy fine sand. This type of soil contains iron-rich clayey deposits as parent material and are commonly found in interfluvial lands (Natural Resources Conservation Service, 2019). The weather reported from KLAHOMER3, a Weather Underground Company station, indicated that the highest accumulate precipitation from October 2017 to September 2020, occurred in February

2017 with 299.7 mm and the lowest was measured in September 2019 with 9.9 mm. Additionally, the highest temperature was reported in August 2019 and the lowest reported temperature was in January 2017 with 4.7°C. The average annual precipitation in Homer, LA is 1,419.4 mm with an annual high temperature of 24°C and annual low temperature of 10°C.

Table 2.3. Weather description from 2017-2020 for Hill Farm Station, Homer, LA.

Month	Monthly accumulate precipitation. (mm)				Average monthly air temperature (°C)		
	2017-2018	2018-2019	2019-2020		2017-2018	2018-2019	2019-2020
Oct.	18.8	153.4	202.4		17.8	18.7	17.9
Nov.	38.1	119.4	29.2		13.7	9.3	10.3
Dec.	114.3	332.2	66.3		7.5	8.3	9.2
Jan.	78.0	152.9	183.4		4.7	6.8	9.3
Feb.	299.7	124.5	195.1		9.5	10.7	9.6
Mar.	168.4	89.7	189.5		14.5	11.0	16.6
Apr.	113.3	276.4	145.0		15.1	17.7	17.8
May	22.6	196.9	113.5		24.6	23.5	22.3
Jun.	80.0	226.8	105.9		27.2	26.2	27.1
Jul.	24.9	94.2	164.1		28.1	28.3	28.9
Aug.	104.1	51.3	108.5		24.1	29.4	26.9
Sep.	104.1	9.9	127.0		24.1	28.2	23.6
Total	1166.3	1827.6	1629.9	Mean	17.6	18.2	18.3

Source: (Weather Underground, 2021).

Cool-season annuals (see Section 2.1 for rates) were not fertilized, however the bahiagrass was fertilized utilizing phosphorus (0-46-0) and potash (0-0-60) fertilizers applied at 168 kg $ha^{-}1$ and 33.6 kg ha^{-1}, respectively in November 2018. Additional fertilizer applications of 33-0-0-12 (N-P-K-S) was applied at 37 kg ha^{-1} in May, June, and July of each year starting in 2018.

2.2. Soil sampling

Soils samples were collected prior to or at initial planting of winter annual cover crops (October 2017), after the first growing season (April-May 2018) for Brown Loam and Ben Hur only, and at the conclusion of the study (May 2020). Samples collected in 2018 and 2020 were taken in late spring to observe the effect of these annuals on the soil when warm-season pastures were finishing their dormancy season. Soils samples were taken in each plot utilizing six soil cores (7 cm diameter) at two depths, 0-10 cm and 10-20 cm depth. To have a better representation of the soil area, cores were mixed and subsamples for biological and chemical analysis were collected in the field.

2.3. Chemical and Biological analysis

Soil parameters were assessed using measures of soil nutrient availability, SOM, total N and C, soil respiration, inorganic N, soil microbial activity (via enzymatic assays and soil respiration) and microbial community structure (via fatty acid profiles). Upon collection, soil samples for biological analyses were placed in a cooler for transport to the laboratory where they were stored at 4°C until hand sieved to pass through a 4.75 mm sieve. For soil respiration, inorganic N, and enzyme assays subsamples were air-dried for 3 days at room temperature. Subsamples for nutrient analysis were submitted to the Soil Testing and Plant Analysis lab at LSU (Table 2.4).

Table 2.4. Soil nutrient availability, soil pH, total N, and total C were measure at the Soil Testing and Plant Analysis Laboratory at Louisiana State University.

Soil Test	Extractant	Conditions	Analysis	Reference
Phosphorus, Potassium, Calcium, Magnesium, Sodium, Sulfur, Copper, Zinc	Mehlich 3	2 g soil / 20 mL solution, 5 min. shaking. (3.75 M NH_4F – 0.25 M EDTA NH_4NO_3, CH_3COOH, and HNO_3)	ICP	Mehlich, 1984
pH	Water	10 g soil / 10 mL deionized H_2O 2 hr. equilibration	pH meter + electrode	McLean, 1982
Nitrogen		0.25 g soil	LECO CN Analyzer	Dumas Dry-Combustion
Carbon		0.25 g soil	LECO CN Analyzer	Dumas Dry-Combustion

Source: (Soil Testing & Plant Analysis lab LSU AgCenter, 2021).

Whole SOM was determined using percent Loss-on-Ignition method on 5 g oven dried (105°C for 18 hours) samples maintained at 400°C for 24 hrs (Nelson and Sommers, 1996). Percent Loss-on-Ignition was calculated as:

$$\% \text{ LOI} = ((\text{Weight105} - \text{Weight400})/\text{Weight105}) * 100$$

Inorganic N concentrations were measured based on 2 *M* KCl solution extraction procedure, 1 g air-dried soil (sieved to <4.75 mm) samples were placed in a 15 mL centrifuge tube with 10 mL of 2 *M* KCl and shaken for 45 minutes. After shaking, samples were filtered using Whatman No. 42 filter paper and extracted for ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N). The NO_3^--N concentration was measured using a vanadium chloride solution and for NH_4^+-N, a salicylate solution as described by Hood-Nowotny et al. (2010). After incubation (1 hr at 37°C for NO_3^--N and 1-hr at room temp for NH_4^+-N) absorbance data was measured using an EON spectrophotometer BioTek at 540 nm and 650 nm for NO_3^--N and NH_4^+-N, respectively.

Enzyme activities were assessed as described by Tabatabi (1994) and Parham and Deng (2000). Briefly 0.5 g of air-dried sieved soil was incubated with the appropriate buffer and substrate. An additional subsample was incubated without the substrate to serve as a control. Following incubation samples were filtered and absorbance measured using an EON Microplate Reader (BioTek, Vermont, USA). Enzyme activity was determined based on a calibration curve established using the appropriate substrate.

Microbial community structure was determined using ester linked FAME analysis (Shutter and Dick, 2000). Field moist soil samples went through a methylation process in which 0.2 M KOH was added in methanol and subjected to a 37°C water bath for 60 minutes, vortexing every 15 minutes four times. Then, samples were neutralized by addition of 1.0 M acetic acid and vortexed again. Samples were extracted with hexane solution previously inverted and centrifuged 5 minutes at 2200 rpm. The organic phase was moved to test tubes and dried utilizing N_2 gas at 37°C. Lastly, samples were rehydrated with hexane and an internal standard and run using a then detected on and gas chromatograph (Agilent Technologies 7890B GC) with a flame ionization detector (FID). Biomarkers were used for identification of soil microbial groups, this included 17:0 10-methyl for actinomycetes (Actino), 16:1 w5c for arbuscular mycorrhizal fungi (AMF), 18:3 w6c, 18:1 w9c for saprophytic fungi, 16:1 w9c, 16:1 w7c, 19:0 cyclo w6c, 18:1 w7c for Gram negative (GMn) bacteria, and 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:0 anteiso, 17:0 iso, 17:1 w9c, 18:0 for Gram positive (GMp) bacteria (Frostegård and Bååth, 1996; Kroppenstedt, 1992; Madan et al., 2002; Walling et al., 1996; Zak et al., 1996; Zelles, 1997; Zogg et al., 1997).

Analysis of variance (ANOVA) were determined using R statistical software (R Core Team, 2013). Soil depth, year of sampling, cool-season annuals overseeded, and harvest frequency treatments were considered independent variables. Using the *vegan* package in R (Oksanen et al., 2020), analyses of FAME data was performed using a correlation matrix. *Vegan* package contains the methods of multivariate analysis needed for analyze ecological communities and tools for diversity analysis. ANOVAs of soil nutrient variable was determined using the lm function in R. For multiple comparisons and a grouping of treatments LSD-test function in *agricolae* package was used. The level by alpha default used was 0.05.

Chapter 3. Results

3.1. Soil chemical properties

3.1.1. Ben Hur

Soil organic matter (*P≤0.0001*) along with TC (*P≤0.0001*), TN (*P≤0.0001*), and NH_4^+-N (*P=0.00017*) increased with depth while pH (*P≤0.0001*) was highest at 10-20 cm (Table 3.1). Over time SOM (*P=0.001*) significantly increased by 47% from year 2017 to 2020 (Table 3.2) at the Ben Hur location. Also, NH_4^+-N increased by 65% from 2017 to 2020 (Table 3.2). However, in the case of NO_3^--N from 2017 to 2018 an increase of 40% was observed but in 2020 it decreased by 167%. The pH increased by 2% from 2017 to 2018 but decreased by 5% in 2020 (Table 3.2).

Table. 3.1. Soil organic matter and nutrient concentration between depth within soil properties.

Depth	TC	TN	NH_4^+-N	SOM
	mg kg^{-1}			g kg^{-1}
0-10cm	34,979a	4,034a	15.01a	49.22a
10-20cm	15,743b	1,901b	12.28b	24.90b

Lowercase letters denote difference between depth within soil properties. TC= total carbon, TN=total nitrogen, NH_4^+-N= nitrate-N, SOM= soil organic matter.

Table. 3.2. Nutrient concentration over time at 0-20 cm depth.

Year	NO_3^--N	NH_4^+-N	TC	TN	SOM	pH
	mg kg^{-1}				g kg^{-1}	
2017	22.75b	5.94c	23,867a	2,941a	30.40c	6.44
2018	33.99a	7.28b	26,976a	2,931a	35.00b	6.57
2020	3.08c	11.65a	25,327a	3,040a	49.20a	6.23
p-values	*≤0.0001*	*0.0002*	*0.0501*	*0.738*	*0.001*	*≤0.0001*

Lowercase letters denote difference overtime within soil properties. NO_3^--N= nitrate-N, NH_4^+-N= ammonium-N, TC= total carbon, TN=total nitrogen, SOM= soil organic matter.

Forage harvest frequency was a factor affecting NO_3^--N (*P=0.0048*), and NH_4^+-N (P *P≤0.0001*) based on the average data from 2017 to 2020. In the case of SOM was not significantly affected (*P= 0.8287*) by forage harvest frequency, however SOM content did vary between treatments. The

8-week harvest frequency treatment tended to have lower SOM with 34.1 g kg^{-1} compared to the mulching (35 g kg^{-1}) and 4-week harvest frequency with 34.3 g kg^{-1}. A different response was measured for NO_3^--N and NH_4^+-N. The 8-week harvest frequency treatment have greater NO_3^--N with 27.4 mg kg^{-1} compared to the mulching (20.8 mg kg^{-1}) and 4-week harvest frequency with 21.5 mg kg^{-1}. The 4-week harvest frequency treatment tended to have lower NH_4^+-N with 10.8 mg kg^{-1} compared to the mulching (14.8 mg kg^{-1}) and 8-week harvest frequency with 15.2 mg kg^{-1} (Table 3.3).

Table. 3.3. Nitrate-N (NO_3^--N), ammonium-N (NH_4^+-N), and soil organic matter (SOM) under harvest frequency treatments.

Harvest frequency	NO_3^--N	NH_4^+-N	SOM
	mg kg^{-1}		g kg^{-1}
4-week	21.54b	10.84b	34.27a
8-week	27.37a	15.18a	34.09a
Mulching	20.79b	14.85a	34.92a

Lowercase letters denote difference between harvest frequency treatments within soil properties.

The overseeding treatment did not present a significant effect on SOM overall, however SOM in check tended to be lower compared to the rest of the overseeding treatments. In 2020, the highest SOM was reported in legumes monoculture overseeded treatment and the lowest SOM reported was under the check with a SOM. Total N was not significantly affected by overseeded treatment, but same as SOM presented lower concentrations in the check compared to the rest of the overseeding treatments (Table 3.4). On the other hand, TC means were different between overseeding treatments and check. For TC, the greater concentration was under the annual ryegrass treatment, but it was not different from 10-mix, and brassica and the check was the lowest compared to the rest of cool-season annuals treatments. For TN, while not significantly different the greater concentration tended to be under the 10-mix treatment.

Table 3.4. Nutrient concentration and SOM under overseeding treatments measured in 2020.

Seeding	NH_4^+-N	NO_3^--N	TC	TN	SOM
	mg kg^{-1}				g kg^{-1}
Check	11.60a	3.34a	21,648d	2,705a	37.15a
Brassica	11.25a	3.19a	25,218ab	3,068a	39.23a
Grass	11.58a	3.16a	25,160b	3,085a	39.87a
Legume	11.35a	2.82a	24,791c	2,981a	48.46a
Ryegrass	11.59a	3.21a	27,902a	3,180a	38.32a
10-mix	12.53a	2.74a	27,239a	3,225a	40.92a
p-value	*0.5863*	*0.9881*	*0.0155*	*0.3053*	*0.4322*

Lowercase letters denote difference between overseeding within soil properties. NH_4^+-N= ammonium-N, NO_3^--N= nitrate-N, TC= total carbon, TN=total nitrogen, SOM= soil organic matter.

Phosphorus (*P≤0.0001*) and K (*P≤0.0001)* at 0-20 cm depth changed over time. Phosphorus significantly increased from 2017 (17.94 mg kg^{-1}) to 2018 (25.12 mg kg^{-1}) but decrease in 2020 (15.85 mg kg^{-1}). On the other hand, K significantly from 2017 (186.76 mg kg^{-1}) to 2018 (215.02 mg kg^{-1}) but decrease in 2020 (144.70 mg kg^{-1}). Also, P (*P≤0.0001*) was significantly greater in the top depth (0-10 cm) with 20.02 mg kg^{-1} compared to the bottom depth (10-20 cm) with 11.61 mg kg^{-1}. Averaged over the 3 years, P was affected by harvest frequency (*P=0.0011*) with the 4-week harvest frequency resulted in higher P (22.29 mg kg^{-1}). Concentrations of P decreased in 8-week harvest frequency (19.66 mg kg^{-1}) followed by the mulching treatment (17.02 mg kg^{-1}). Conversely, for K mulching presented the second greatest concentration within the harvest frequency treatments followed by the 8-week harvest frequency.

Table 3.5. Interaction of forage harvest frequency and cool-season annuals for calcium (Ca), phosphorus (P), and sodium (Na) in 2020.

	Ca	P	Na	Ca	P	Na	Ca	P	Na
	mg kg^{-1}								
	4-week			8-week			Mulching		
Check	3,759abc	12.8bcd	104.1a	3,516c	20.4b	56.2cdef	3,786abc	9.5d	91.8abc
10-mix	3,752abc	17.7bcd	63.8bcdef	3,811abc	10.9cd	90.9abcd	4,058a	12.7bcd	87.9abcd
Brassica	3,504c	19.9bc	53.9bcdef	3,621bc	14.5bcd	50.3ef	3,866abc	14.5bcd	99.1ab
Grass	3,731abc	17.7bcd	66.8bcdef	3,966ab	13.5bcd	78.9abcdef	3,474c	19.1bc	46.1f
Legume	3,898abc	16.1bcd	61.1cdef	3,759abc	14.2bcd	68.2abcdef	3,835abc	11.7bcd	84.4abcde
Ryegrass	3481c	17.4bcd	47.5f	3713abc	30.1a	49.7ef	3973ab	13.5bcd	79.4abcdef

Lowercase letters denote significant difference between overseeding treatments within soil property.

There was a significant impact of harvest frequency treatments and cool-season annuals overseeded on Ca (*P= 0.04603*), P (*P=0.008455*), and Na (*P=0.040529*). The 10-mix overseeded resulted in greater Ca concentrations under mulching treatment increasing 8% and 6% compared to 4-week and 8-week harvest frequency treatments, respectively (Table 3.5). The brassica function group plots contained greater Ca concentration in soil under the mulching treatment increasing 10% and 7% compared to 4-week and 8-week harvest frequency, respectively. Under the same plots, soil P was 31% greater following the 4-week harvest than that found under 8-week and mulching treatments. Soil Na was 59% and 65% greater under mulching than the 4-week and 8-week harvest interval treatments, respectively (Table 3.5).

Soil Ca in plots overseeded with the grass functional group was 6% and 13% higher under 8-week harvest frequency treatment compared to 4-week and mulching harvest frequency treatments, respectively. In the same overseeding treatment, soil P was 8% and 34% greater under mulching than 4-week and 8-week harvest intervals, respectively. Also, following the grass functional group, soil Na increased 17% and 52% at 8-week harvest compared to 4-week and mulching, respectively. On the other hand, Ca concentration decreased 4% under 8-week harvest frequency treatment and

1.6% under mulching compared to 4-week harvest frequency treatment in the plots overseeded with cool-season legumes. The same pattern was observed for soil P under overseeded legumes, with soil P decreasing under 8-week and mulching harvest frequency treatments by 3% and 32% respectively (Table 3.5). In the case of soil Na overseeding legumes, 4-week and 8-week harvest frequency reduced its concentration by 32% and 21% compared to mulching. Mulching increased soil Ca under plots overseeded with annual ryegrass by 13% and 7% in 4-week and 8-week harvest frequency treatments, respectively. Soil P increased under 8-week harvest frequency by 53% and 76% compared to 4-week and mulching harvest frequency treatments, respectively. Still, annual ryegrass overseeded plots resulted in greater soil Na under the mulching treatment which was 50% and 46% higher compared to 4-week and 8-week harvest frequency treatments (Table 3.5).

3.1.2. Brown Loam

Soil organic matter increased by 105% from 2017 to 2020. Total C increased by 10% from 2017 to 2020. Total N did not change from 2017 to 2018 but increased significantly by 17% in 2020. Ammonium-N remained constant through 2018 but had decreased by 67% in 2020. Nitrate-N concentrations decreased over the course of the study, decreasing by 33% from 2017 to 2018 and again by 90% in 2020 (Table 3.6).

Table 3.6. Nutrient concentrations and SOM changes through years.

Year	NO_3^--N	NH_4^+-N	TN	TC	SOM
	mg kg^{-1}		g kg^{-1}		
2017	25.42a	27.19a	1.3b	9.7b	12.8c
2018	20.12a	29.35a	1.2b	10.0b	15.7b
2020	1.77b	9.44b	1.4a	10.6a	26.1a
p-value	≤0.0001	≤0.0001	≤0.0001	0.03177	≤0.0001

Lowercase letters denote significant difference over time within soil property. NO_3^--N= nitrate-N, NH_4^+-N= ammonium-N, TN=total nitrogen, TC= total carbon, SOM= soil organic matter.

There was a significant interaction between depth and sampling year impacting total C *($P\leq0.0001$)*, total N *($P\leq0.0001$)*, NO_3^--N (*$P=0.020150$*), and pH (*$P=0.03279$*). In the 0-10 cm depth total C increased over time by 22%, while in the 10-20 cm depth decreased overtime by 10% (Table 3.7). Total N in the top 10 cm of soil increased an average of 26% and in the bottom depth decreased by 5% from 2017 to 2020. Nitrate-N decreased by 94% over time in the top 10 cm of soil and in 10-20 cm it decreased 92% over time. Even though, pH increased in 2018 it decreased in 2020 for both depths, decreasing by 8% and by 2% overtime in 0-10 cm and 10-20 cm depth, respectively (Table 3.7).

Table 3.7. Interaction of depth and year in total carbon (TC), total nitrogen (TN), ammonium-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N), and pH.

	2017	2018	2020	2017	2018	2020
	0-10cm			10-20cm		
TC (g kg^{-1})	11.7c	13.3b	14.3a	7,8d	6.5e	7.0de
TN (g kg^{-1})	1.4c	1.60b	1.8a	1.1d	1.0e	1.0d
NO_3^--N (g kg^{-1})	29.03a	21.18b	1.79c	21.81b	19.02b	1.74c
pH	5.34d	5.80c	5.28d	6.02b	6.29a	5.89c

Lowercase letters denote significant difference between depth and year within soil properties.

In 2020 P (*$P\leq0.0001$*), K (*$P\leq0.0001$*), and S (*$P\leq0.0001$*) were significantly affected by depth, having greater concentrations in the 0-10 cm depth in all three. Phosphorus decreased from 32.48 mg kg^{-1} in 0-10cm depth to 11.61 mg kg^{-1} in 10-20cm depth. Potassium decreased from 72.44 mg kg^{-1} in 0-10cm depth to 34.66 mg kg^{-1} in 10-20cm depth. Sulfur decreased from 10.54 mg kg^{-1} in 0-10cm depth to 6.59 mg kg^{-1} in 10-20cm depth. Soil P (*$P=0.002$*) and K (*$P=0.005$*) were significantly different between forage harvest frequency treatments. The 4-week harvest interval resulted in greater P concentrations (27 mg kg^{-1}) and 8-week harvest had the lowest P concentration (18 mg kg^{-1}). For K, there was greater concentration measured under mulching treatment (60.5 mg kg^{-1}) and the lower under 8-week (49.9 mg kg^{-1}) but was not very different from 4-week harvest (50.2 mg kg^{-1}).

3.1.3. Hill Farm

Except for pH, chemical properties were greater in 0-10cm depth in average data from 2017 and 2020 (Table 3.8). Also, TN, NO_3^--N, and SOM were changing over time from 2017 to 2020. Total nitrogen was reduced by 7.5% in year 2020 compared to the first year, 2017. The same happened with NO_3^--N which was reduced by 10% in 2020. On the other hand, SOM had increased 57% in 2020. (Table 3.9).

Table 3.8. Nutrient data, SOM, and pH impacted by depth.

Depth (cm)	NO_3^--N	TC	TN	SOM	pH
	mg kg^{-1}		g kg $^{-1}$		
0-10	12.94a	16.5a	1.7a	28.02a	5.342a
0-20	8.59b	7.8b	0.8b	13.57b	5.452b
p-value	*≤0.0001*	*≤0.0001*	*≤0.0001*	*≤0.0001*	*0.00259*

Lowercase letters denote significant difference between depths within soil property. NO_3^--N= nitrate-N, TC= total carbon, TN=total nitrogen, SOM= soil organic matter.

Table 3.9. Inorganic nitrate, total nitrogen, and soil organic matter impacted by time.

Year	NO_3^--N	TN	SOM
	mg kg^{-1}		g kg $^{-1}$
2017	7.26b	1,332a	8.86b
2020	15.02a	1,232b	20.80a
p-value	*≤0.0001*	*0.01586*	*≤0.0001*

Lowercase letters denote significant difference overtime within soil property. NO_3^--N= nitrate-N, TN=total nitrogen, SOM= soil organic matter.

Interaction of depth and year impacted SOM *($P \leq 0.0001$)*. Soil organic matter increased over time in 0-10 cm by 1566% (1.094 in g kg^{-1} in 2017 and 2.802 in g kg^{-1} in 2020) and increased 100% in 10-20 cm (0.677 in g kg^{-1} in 2017 and 1.357 in g kg^{-1} in 2020).

In 2020, there were interactions of harvest frequency treatments and depth significantly impacted Cu (*P= 0.0047*), Na *(P= 0.0004*), and Zn (*P= 0.0018*) concentrations. Measured at 0-10cm depth, Cu increased 26% and 15% in mulching compared to 4-week and 8-week harvest frequency, respectively. At 10-20cm depth, Cu increased 6.24 % and 2.10% at 8-week harvest frequency compared to 4-week and mulching harvest frequency, respectively (Table 3.10). Sodium measured at the 0-10cm depth increased 65% and 50% in mulching treatment compared to 4-week and mulching harvest frequency, respectively (Table 3.10). At 10-20 cm Na increased 44% and 4% under the 4-week harvest frequency compared to 8-week and mulching treatment, respectively. Zinc increased in 8-week at 0-10cm depth and increased at 4-week harvest frequency at 10-20cm depth (Table 3.10).

Table 3.10. Interaction of harvest frequency treatments and depth in copper (Cu), sodium (Na), and zinc (Zn) in 2020.

	4-week	8-week	Mulching	4-week	8-week	Mulching
		0-10cm			10-20cm	
			mg kg^{-1}			
Cu	10.85c	12.21b	14.16b	4.97d	5.29d	5.18d
Na	80.54bc	158.19a	95.33b	58.13cd	37.26d	55.75cd
Zn	16.69b	24.63a	19.82b	6.26c	6.07c	6.18c

Lowercase letters denote significant difference between harvest frequency treatments and by depth within soil property.

3.2. Soil biological properties

3.2.1. Ben-Hur

Potential β-glucosidase activity increased significantly over time, increasing by 74% in 2017 and an additional increase of 18% by 2020. Depth also impacted β-glucosidase activity with increased activity in the top depth (0-10 cm). Potential N-acetyl-β-glucosaminidase (NAGase) activity increased significantly (54%) in 2018 but decreased by 13% in 2020. Despite this decrease, NAGase activity increased 46% by the end of the study in 2020. NAGase activity was 87% higher in the top 10cm compared to the 10-20 cm depth in average over the years from 2017 to 2020 (Table 3.11). Additionally, forage harvest frequency treatments significantly impacted NAGase activity (P= 0.01710). The highest NAGase activity was reported under mulching treatment 56.27 mg p-nitrophenol kg^{-1}soil h^{-1}, followed by the 8-week harvest frequency with 50.77 mg p-nitrophenol kg^{-1}soil h^{-1}, and the lowest activity was measured under 4-week with 43.32 mg p-nitrophenol kg^{-1}soil h^{-1} (Table 3.11).

Table 3.11. β-glucosidase activity and N-acetyl-β-glucosaminidase (NAGase) influenced by time and depth and harvest frequency in average 2017-2020.

Depth (cm)	β-glucosidase	NAGase
	mg p-nitrophenol kg^{-1}soil h^{-1}	
0_10	119.96a	71.42a
10_20	42.59b	27.97b
p-value	*≤0.0001*	*≤0.0001*
Forage harvest frequency	**β-glucosidase**	**NAGase**
	mg p-nitrophenol kg^{-1}soil h^{-1}	
4-week	84.15b	43.32c
8-week	77.28b	50.77b
Mulching	85.85b	56.27a
p-value	*0.6827*	*0.01710*

Lowercase letters denote significant difference between depth and forage harvest frequency within enzymes activity.

There was a significant interaction of depth, harvest frequency, and time on potential NAGAse activity (*P≤0.0001*). Greater NAGase activity was measured at the 0-10cm depth over all three years under the three harvest frequency treatments (Table 3.11). In 2020 at the 0-10 cm depth, the highest NAGase activity was reported under the mulching treatment with a difference of 31% and 9% compared to 4-week and 8-week harvest treatments, respectively. At the 10-20 cm depth, the same pattern was observed, with greater NAGase activity under mulching compared to the 4-week and 8-week harvest frequency treatments (Table 3.12). Measured at 0-20cm depth, NAGase activity was greater in 2018 compared to 2017 and 2020 under 4-week harvest frequency. However, in the case of mulching treatment the greater NAGase activity was measured at 0-10 cm depth in 2020 (Table 3.12). The greatest NAGase activity overall, was reported during 2018 at 0-10 cm depth in a 4-week harvest frequency treatment. On the other hand, the lowest NAGase activity was reported in 2017 at 10-20cm depth in 4-week harvest frequency treatment (Table 3.12).

Table 3.12. Three-way interaction of N-acetyl-β-glucosaminidase (NAGase) (mg p-nitrophenol kg^{-1}soil h^{-1}) in 2020.

	4-week			8-week			Mulching		
Depth(cm)	2017	2018	2020	2017	2018	2020	2017	2018	2020
0-10	20.88gh	107.11a	59.20cd	36.31efg	75.41b	73.85bc	49.13de	73.48bc	81.21b
10-20	16.63h	41.93ef	24.26gh	20.46h	28.11fgh	27.70fgh	18.51h	29.75fgh	31.34fgh

Lowercase letters denote significant difference between depth, over time and harvest frequency in NAGase activity.

There was a significant interaction between cool-season annuals and harvest frequency on potential NAGase activity *(P= 0.04984)*. The greatest NAGase activity was measured when overseeding brassicas and harvesting at an 8-week interval. The lowest NAGase activity was measured when overseeding grasses and harvesting at a 4-week frequency (Table 3.12). At 4-week harvest frequency, greater NAGase activity was measured when legumes were overseeded and the lowest activity was reported when grasses were overseeded into bermudagrass. At the 8-week harvest frequency, greater NAGase activity was measured under overseeded brassicas and lower activity was reported under overseeded legumes in bermudagrass. Under the mulching treatment, the greater NAGase activity was reported following overseeding legumes and the lower activity was measured when annual ryegrass was overseeded in bermudagrass (Table 3.13).

Table 3.13. Interaction of cool-season annuals and forage harvest frequency treatments N-acetyl-β-glucosaminidase (NAGase).

Cool-season annuals	4-week	8-week	Mulching
	mg p-nitrophenol kg^{-1} soil h^{-1}		
Check	43.11de	38.51e	54.53abcde
10-mix	44.4cde	39.06d	55.04abcde
Brassica	40.44de	69.74a	57.87abcd
Grass	34.48e	50.90abcde	57.69abcd
Legume	55.30abcde	38.24de	64.66abc
Ryegrass	44.49cde	68.20ab	47.85bcde

Lowercase letters denote difference between.

There was an interaction of time and depth impacting absolute abundance of protozoa (*$P \leq 0.0001$*), Gram-negative bacteria (GMn) (*$P=0.01344$*), saprophytic fungi (*$P=0.0495$*), and arbuscular mycorrhizal fungi (AMF) (*$P=0.0453$*). Protozoa increased in 2018 and decreased in 2020 in both depths, ultimately decreasing 10% and 14% over time at the 0-10 cm depth and 10-20 cm depth, respectively. Gram positive bacteria increased 42% and 174% by 2020 in 0-10 cm and 10-20 cm depths, respectively. Saprophytic fungi and arbuscular mycorrhiza fungi increased in 0-10cm depth and decreased in 10-20 cm depth (Table 3.14).

Table 3.14. Interaction of absolute abundance of fatty acid methyl ester over time and by depth.

Depth	Protozoa	GMp	Fungi	AMF
	nmols g-1			
0-10 cm				
2017	0.77b	11.95ab	10.49a	5.00a
2018	1.48a	17.98a	8.64a	1.40d
2020	0.69b	20.57abc	9.92a	3.41a
10-20 cm				
2017	0.36c	6.8bc	5.27b	3.19b
2018	1.4a	15.92abc	5.78b	0.84d
2020	0.31c	18.63c	4.75b	2.14c

GMp=Gram positive bacteria; Fungi=saprophytic fungi; AMF=arbuscular mycorrhizal fungi. Lowercase letters denote difference between depth, harvest frequency, and over time.

Over time the relative abundance of GMn along with, relative abundance of AMF, fungi to bacteria ratio (F:B), total bacteria and protozoa increased (Fig. 3.1). Relative abundance of total bacteria, Gram positive bacteria, saprophytic fungi, actinomycetes, and fungi to bacteria ratio, increased in the top depth (Figure 3.2). Measured at 10-20 cm depth, AMF and GMn relative abundance increased (Figure 3.2).

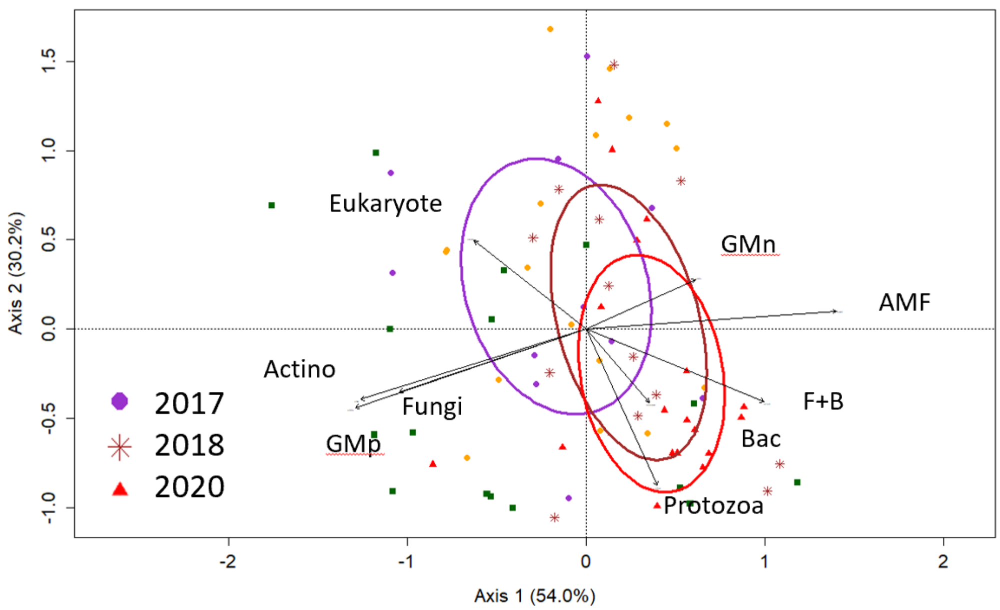

Figure 3.1. Principle coordinate analyses of relative abundance of fatty acid methyl ester data over time. GMp=Gram positive bacteria; GMn=Gram negative bacteria; AMF= arbuscular mycorrhizal fungi; Fungi=saprophytic fungi; Bac=total bacteria, F:B= fungi to bacteria ratio.

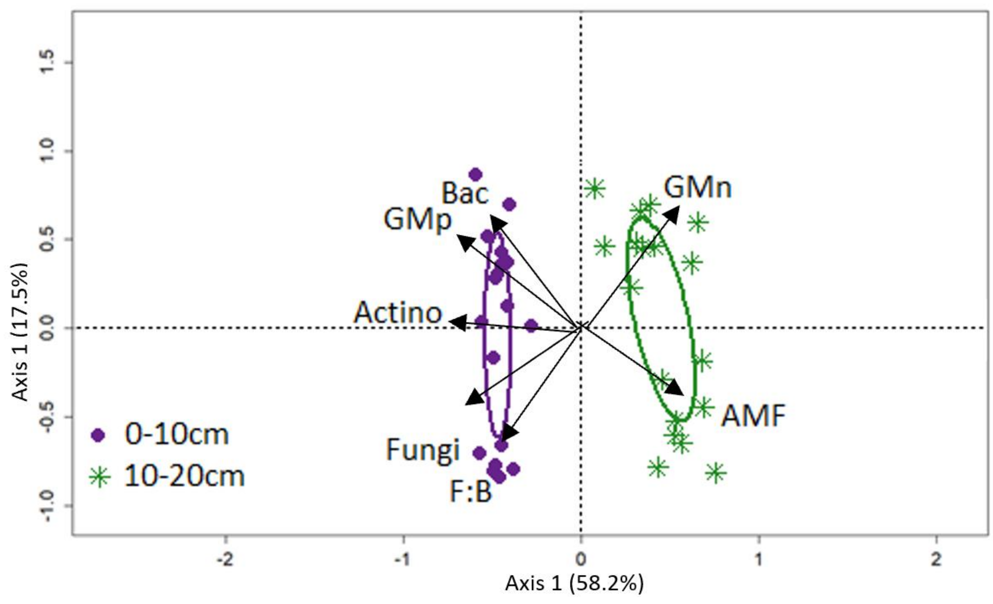

Figure 3.2. Principle coordinate Analyses of relative abundance of fatty acid methyl ester data at 0-10cm and 10-20 cm depth. GMp=Gram positive bacteria; GMn=Gram negative bacteria; AMF=arbuscular mycorrhizal fungi; Fungi=saprophytic fungi; Bac=total bacteria, F:B= fungi to bacteria ratio.

3.2.2. Brown Loam

There was an impact on NAGase activity (*$P \leq 0.0001$*) over time, depth, and harvest frequency treatments. Looking into individual harvest frequency treatments, all of them reported increased NAGase activity over time at both depths, except in samples collected at 0-10 cm under the mulching treatment which decreased 0.7% from 2018 to 2020 (Table 3.15). Overall, greater NAGase activity was measured in the top 10 cm under all harvest frequency treatments from 2017 to 2020 (Table 3.15).

Table 3.15. Three-way interaction of N-acetyl-β-glucosaminidase (NAGase).

	4-week			8-week			Mulching		
	2017	2018	2020	2017	2018	2020	2017	2018	2020
	mg p-nitrophenol kg^{-1}soil h^{-1}								
0-10 cm	13.10de	30.19b	38.27a	17.66cd	38.17a	41.55a	15.34de	30.97b	30.77b
10-20 cm	12.65de	13.50de	16.06de	14.70de	15.38de	16.31de	10.99e	13.80de	22.12c

Lowercase letters denote difference over time, harvest frequency, and depth.

Forage harvest frequency treatments and time impacted potential β-glucosidase activity *(P= 0.00785*). For all harvest frequency treatments β-glucosidase activity increased over time (Table 3.16). In 2017 and 2018, the 8-week harvest frequency treatment resulted in greater β-glucosidase activity compared to the other harvest frequency treatments. However, in 2020 β-glucosidase activity responded differently to harvest frequency treatment. Mulching resulted in greater β-glucosidase activity compared to the 4- and 8-week harvest frequency treatments (Table 3.16).

Table 3.16. Interaction of forage harvest frequency treatments and year in β-glucosidase activity.

Year	4-week	8-week	Mulching
	mg p-nitrophenol kg^{-1}soil h^{-1}		
2017	1.10b	2.05b	1.63c
2018	1.97c	2.24b	2.06b
2020	3.78a	3.59a	4.24a

Lowercase letters denote significant differences within harvest frequency over time.

The absolute abundance of total FAMEs (*P≤0.0001*), GMp (*P=0.0003*), GMn (*P=0.0002*), Protozoa (*P≤0.0001*), AMF (*P≤0.0001*), total bacteria *(P=0.0008)*, and eukaryotes (*P≤0.0001*) was impacted over time. All these groups increased in 2018 but decreased in 2020, except for AMF decreased in absolute abundance in 2018 but increased in 2020 (Table 3.16). Even though, total FAMEs decreased 25% in 2020 compared to 2018, overall total FAMEs it increased 7% compared to 2017 (Table 3.17).

Table 3.17. Absolute abundance of fatty acid methyl ester over time.

	TF	GMp	GMn	Protozoa	AMF	Bac	Euka
			nmol g^{-1}				
2017	141.28c	7.33b	5.11b	0.44c	1.48b	14.77b	1.09b
2018	200.15a	18.75a	9.12a	1.67a	0.53c	30.17a	4.58a
2020	150.93b	6.16b	5.04b	0.58b	2.21a	13.65c	1.07b

TF= total FAMEs; GMp= Gram positive bacteria; GMn= Gram negative bacteria; AMF= arbuscular mycorrhizal fungi; Bac= total bacteria; Eukary= eukaryotes. Lowercase letters denote difference over time.

In 2017 the relative abundance of actinomycetes, GMp, and saprophytic fungi dominanted the microbial community. By 2018, the population had shifted to a predominance of eukaryotes and shifted further to a predominance of GMn, AMF, F+B, total bacteria, and protozoa in 2020 (Fig. 3.3).

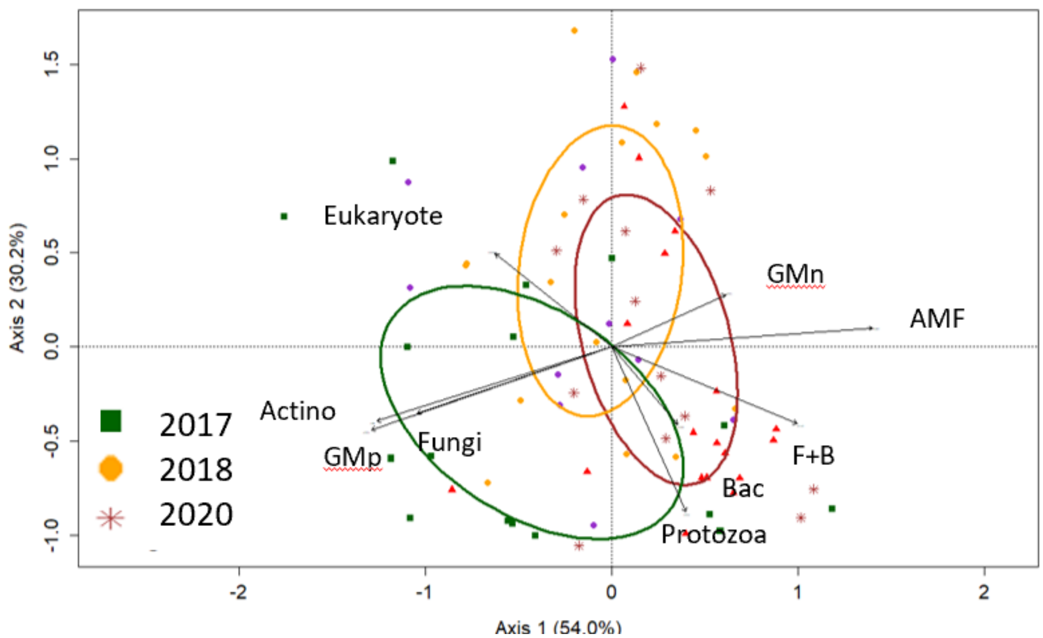

Figure 3.3. Principle coordinate analyses of relative abundance of fatty acid methyl ester data over time. GMp=Gram positive bacteria; GMn=Gram negative bacteria; AMF=arbuscular mycorrhizal fungi; Fungi=saprophytic fungi; Bac=total bacteria, F+B= fungi to bacteria ratio.

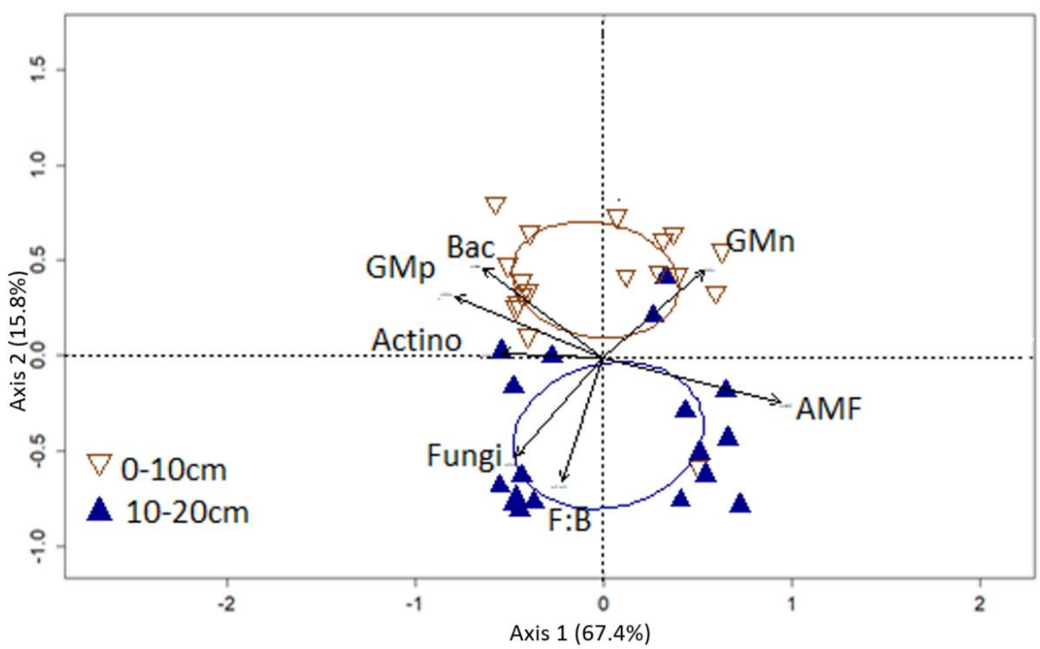

Figure 3.4. Principle coordinate analyses of relative abundance of fatty acid methyl ester data at 0-10cm and 10-20 cm depth. GMp=Gram positive bacteria; GMn=Gram negative bacteria; AMF=arbuscular mycorrhizal fungi; Fungi=saprophytic fungi; Bac=total bacteria; F:B= fungi to bacteria ratio.

3.2.3. Hill Farm

Potential β-glucosidase activity (*$P \leq 0.0001$*) measured in 2020 was significantly higher in the 0-10 cm depth with 37.37 mg p-nitrophenol kg^{-1}soil h^{-1} compared to 10-20cm depth with 15.55 mg p-nitrophenol kg^{-1}soil h^{-1}. Potential NAGase activity (*$P=0.000914$*) was impacted by a three-way interaction of harvest frequency treatments, depth, and year. For all the harvest frequency treatments, greater NAGase activity was measured in the top 10 cm of the soil profile. At the 0-10 cm depth, NAGase activity increased over time in all harvest frequency treatments (Table 3.18). However, at 10-20cm depth NAGase activity decreased in 2020 for all harvest frequency treatments, except 4-week harvest frequency treatment. In 2020 measured at 0-10cm depth, mulching treatment reported the greatest potential NAGase activity (Table 3.18). Overall, mulching harvest frequency treatment reported greater NAGase activity through the harvest frequency treatments (Table 3.18).

Table 3.18. Three-way interaction of harvest frequency, depth, and year in potential N-acetyl-β-glucosaminidase (NAGase) activity.

	NAGase (mg p-nitrophenol kg^{-1}soil h^{-1})					
	2017	2020	2017	2020	2017	2020
Depth	4-week		8-week		Mulching	
0-10cm	19.87de	27.34b	17.81efg	23.92c	20.71d	36.20a
10-20cm	14.52fg	15.65fg	16.94efg	16.60efg	19.20de	12.96h

Lowercase letters denote difference between forage harvest frequency, depth, and year.

Cool-season annuals as a treatment significantly impacted NAGase activity (*$P \leq 0.0001$*) measured at 0-20cm depth in 2020. Overseeding legumes, ryegrass, grass, and brassicas increase potential NAGase activity compared to 10-mix, check, legume and brassica mixture, grass and legume mixture, and grass and brassica mixture (Table 3.19).

Table 3.19. Cool-season annuals impacted N-acetyl-β-glucosaminidase (NAGase) activity in 2020.

NAGase (mg p-nitrophenol kg-1soil h-1)								
Check	Brassica	Grass	Legume	Ryegrass	G+B	G+L	L+B	10-mix
6.09c	19.11ab	21.61a	23.88a	22.18a	3.53c	5.37c	5.97c	11.72bc

G+B= grass and brassica mixtures; G+L= grass and legumes mixtures; L+B= legumes and brassica mixtures. Lowercase letters denote difference between overseeding treatments.

Absolute abundance of GMp (*P= 0.0122*), GMn (*P= 0.0108)*, Fungi (*P= 0.0359)*, total bacteria (*P= 0.025218)*, eukaryote (*P≤0.0001*), and protozoa (*P= 0.0415*) were significantly impacted by time, increasing in 2020 in all cases (Table 3.20). Absolute abundance of AMF (*P≤0.0001*) and fungi to bacteria ratio (*P≤0.0001*) were significantly impacted by an interaction of depth and forage harvest frequency treatments. Measured at 0-10cm depth, AMF increased 189% over time in 4-week harvest frequency treatment while in the 8-week harvest treatments populations decreased and no change was measured under the mulching treatment. For the bottom depth, AMF absolute abundance increased over time in all cases. On the other hand, fungi to bacteria ratio increased over time in the top depth for all harvest frequency treatments (Table 3.21).

Table 3.20. Absolute abundance of fatty acid methyl ester over time.

	GMp	GMn	Fungi	Total Bac	Eukary	Protozoa
			nmol g^{-1}			
2017	7.12b	5.43b	6.20b	14.82b	1.88b	0.91b
2020	12.48a	7.04a	7.65a	21.93a	2.97a	1.30a

GMp=Gram positive bacteria; GMn=Gram negative bacteria; Fungi=saprophytic fungi; Total Bac= total bacteria; Eukary= eukaryotic. Lowercase letters denote difference over time.

Table 3.21. Interaction of forage harvest frequency and year in absolute abundance of fatty acid methyl ester.

	AMF		F:B	
	2017	2020	2017	2020
	nmol g^{-1}			
0-10cm				
4-week	1.66cd	4.79a	0.56abc	0.58c
8-week	2.61b	1.47cde	0.50bc	0.73a
Mulching	1.37cde	1.37cde	0.54abc	0.56abc
10-20cm				
4-week	1.21cde	2.09bc	0.58abc	0.38c
8-week	1.16cde	0.91de	0.48bc	0.75a
Mulching	0.68e	0.89de	0.38c	0.65ab

AMF=arbuscular mycorrhizal fungi; F: B=fungi to bacteria ratio. Lowercase letters denote difference between harvest frequency treatments and depth.

The relative abundance of protozoa and AMF increased under the 4-week harvest frequency treatment, while saprophytic fungi, GMn, and fungi to bacteria ratio increased under the 8-week harvest frequency treatment. The mulching treatment increased the relative abundance of eukaryotes, actinomycetes, total bacteria and GMp bacteria (Figure 3.5). Relative abundance over time reported increased AMF and GMn, and fungi to bacteria ratio (Figure 3.6).

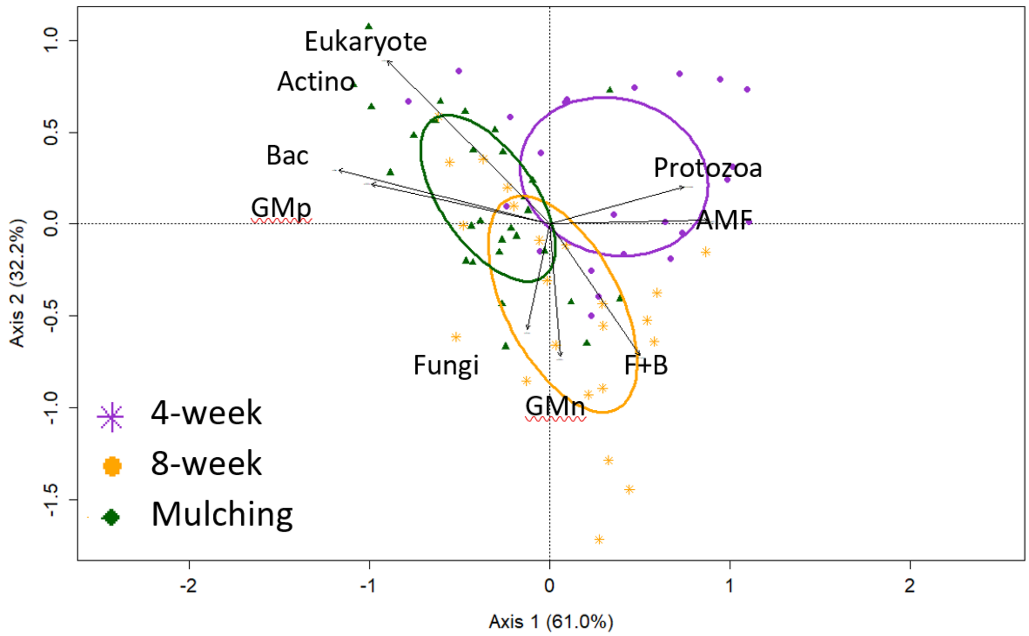

Figure 3.5. Principle coordinate analyses of relative abundance of fatty acid methyl ester data influenced by harvest frequency treatments. Actino= actinomycetes; AMF=arbuscular mycorrhizal fungi; Bac= total bacteria; GMp=Gram positive bacteria; GMn=Gram negative bacteria; Fungi=saprophytic fungi.

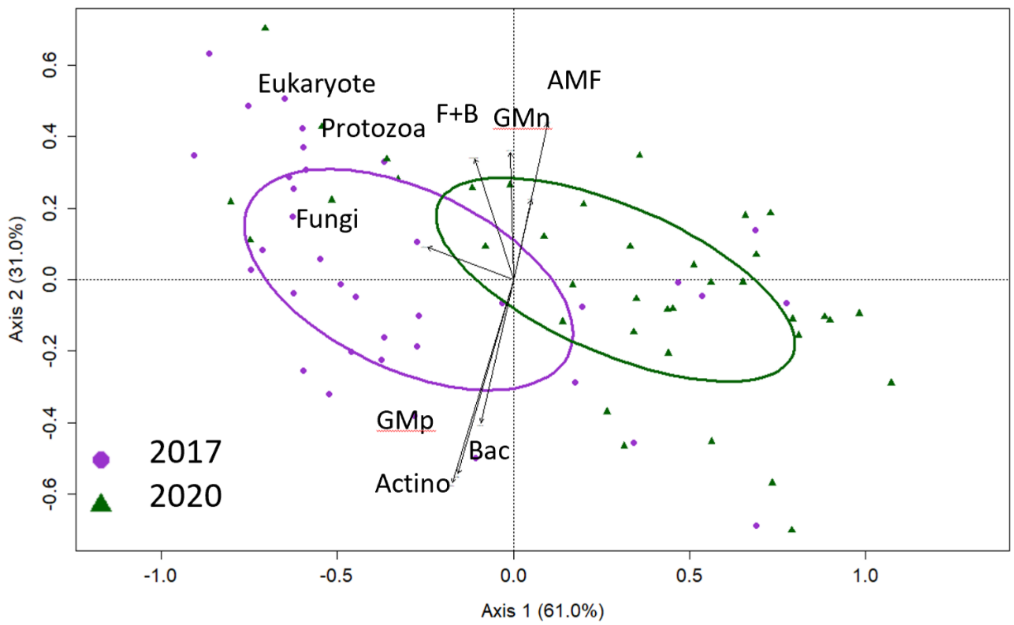

Figure 3.6. Principle coordinate analyses of relative abundance of fatty acid methyl ester data influenced by time. GMp=Gram positive bacteria; GMn=Gram negative bacteria; AMF=arbuscularmycorrhizal fungi; Fungi=saprophytic fungi; Bac=total bacteria, F+B= fungi to bacteria ratio.

3.3. Forage yield

There was a three-way interaction of year, harvest frequency, and cool-season annuals on warm-season annuals forage yield in Ben Hur presenting significant increases in forage yield over time (Table 3.22). Across cool-season annuals in 2018 the forage yield obtained in 10-mix plots was significantly greater than in plots with brassicas, legumes and brassicas, and annual ryegrass. However, this was not the case in 4-week and mulching harvest frequency treatments (Table 3.22).

In 2019, under 8-week harvest frequency treatment, the forage yield in plots with 10-mix was significantly greater compared to brassicas. In 2020, under 4-week harvest frequency, forage yield in plots where annual ryegrass, legume and brassica, grass and brassica, and the control were significantly greater than forage yield in 10-mix plots. In 8-week harvest frequency, only forage yield in 10 mix plots was greater than forage yield in brassicas. In the mulching treatment, all cool-season annuals including the check presented greater warm-season perennial forage yield than in plots where brassicas were planted (Table 3.22).

In Brown Loam, forage yield was greater in 2020 compared to 2018 and 2019. Additionally, for 2020 and 2018 the greater forage yield was found under the mulch treatment, the same with 2019 but 4-week and mulching were greater than 8-week harvest frequency. An interaction of forage harvest frequency, cool season annuals, and year presented increases over time in the three harvest frequency treatments, 2020 presented greater forage yield compared to 2018 and 2020 (Table 3.23). Also, in 2020 forage yield in plots overseeded with legumes, legumes and brassica, and 10-mix species were greater than the rest of cool-season annuals including the check (Table 3.24).

Hill Farm presented significant interaction of harvest frequency and year in forage yield of warm-season perennial pastures, indicating in 2020 they increase significantly compared to 2019 and

2018. Additionally, in 2020 mulch presented the greater forage yield compared to 4-week and 8-week, same as in 2019, in 2018 there were no significant differences between them (Table 3.25).

Table 3.22. Three-way interaction of year, harvest frequency and cool-season annuals treatments with warm-season perennial bermudagrass forage mass in Ben Hur.

	Year								
	2018			2019			2020		
	Harvest Frequency								
Cool-season annuals	4-week	8-week	Mulching	4-week	8-week	Mulching	4-week	8-week	Mulching
	- Mg ha^{-1} -								
10-mix	8.25aB	10.17aB	7.81aB	8.16aB	9.31aB	7.65aB	14.01dA	17.02aA	15.20aA
Grasses	8.06aB	7.87abcB	6.90aB	8.32aB	8.52abB	8.83aB	15.76abcdA	15.36abA	16.34aA
Legumes	7.56aB	8.18abcB	7.99aB	7.84aB	7.28abB	7.66aB	14.66cdA	16.97abA	16.96aA
Brassicas	7.69aB	6.09cB	7.15aB	7.66aB	6.19bB	6.82aB	16.01abcdA	13.93bA	8.24bB
Grass-Legume	8.35aB	8.50abcB	6.40aB	8.07aB	7.72abB	7.05aB	15.01bcdA	15.04abA	15.49aA
Grass-Brassica	7.34aBC	9.93abB	7.77aBC	7.57aBC	8.33abBC	6.37aC	17.67abcA	16.01abA	14.69aA
Legume-Brassica	8.34aB	6.89bcB	8.57aB	7.89aB	7.90abB	8.22aB	17.93abA	16.90abA	14.98aA
Annual Ryegrass	7.03aB	6.83cB	6.35aB	7.53aB	7.34abB	7.32aB	18.18aA	15.64abA	15.47aA
Check	7.63aB	7.17abcB	8.39aB	8.16aB	8.00abB	8.89aB	17.61abcA	17.06aA	16.53aA

Lowercase letters denote difference within columns and upper-case letters denote difference within rows. Same letters means there is no differences.

Table 3.23. Interaction of harvest frequency and year with warm-season perennial bahiagrass forage mass in Brown Loam.

	Year		
Harvest Frequency	2018	2019	2020
	- - - - - - - - - - -	Mg ha^{-1} - - - - - - - - - - -	
4-week	2.87bC	3.77aB	6.29bA
8-week	3.11bC	3.51bB	6.18bA
Mulching	3.36aC	3.82aB	6.70aA

Lowercase letters denote difference within columns and upper-case letters denote difference within rows. Same letters means there is no differences.

Table 3.24. Interaction of year and cool-season annuals with warm-season perennial bahiagrass forage mass in Brown Loam.

Cool-season annuals	2018	2019	2020
	- - - - - - - - - - -	Mg ha^{-1} - - - - - - - - - - -	
10-mix	3.05aC	3.63aB	6.72abA
Grasses	3.19aB	3.59aB	6.20cA
Legumes	3.15aC	3.63aB	6.96aA
Brassicas	2.99aC	3.49aB	6.20cA
Grass-Legume	3.32aB	3.72aB	6.36bcA
Grass-Brassica	3.23aC	3.73aB	6.10cdA
Legume-Brassica	3.01aC	3.92aB	7.06aA
Annual Ryegrass	3.07aC	3.60aB	6.11cdA
Check	3.05aC	3.77aB	6.50bcA

Lowercase letters denote difference within columns and upper-case letters denote difference within rows. Same letters means there is no differences.

Table 3.25. Interaction of harvest frequency and year with warm-season perennial bahiagrass forage mass in Hill Farm.

	Year		
Harvest Frequency	2018	2019	2020
	- - - - - - - - - - - - - - Mg ha^{-1} - - - - - - - - - - - - - -		
4-week	3.13aC	5.52bB	6.15bA
8-week	2.90aB	5.60bA	5.64cA
Mulching	3.06aC	6.19aB	6.79aA

Lowercase letters denote difference within columns and upper-case letters denote difference within rows. Same letters means there is no differences.

Chapter 4. Discussion

Changes over time were observed for several soil properties found in overseeded plots and the check, suggesting that these improvements were not specifically related to the overseeding of winter annuals. However, these improvements may be associated with the benefits that perennial pastures provide to soil including contribution to soil microbial biomass, diversity, and addition of more organic residues into the soil (Dhakal & Islam, 2019). Further, cool-season annuals were not a factor affecting many soil properties, except for TC in Ben Hur which was the only soil property directly affected by overseeding cool-season annuals. It is important to mention that some winter annuals treatments in Brown Loam and Ben Hur were not well established possibly impacting their influence on soil properties. Total C was significantly greater when overseeding cool-season annuals compared to control, with the greatest concentration of TC measured when overseeding annual ryegrass at Ben Hur. Additionally, reduced NAGase activity in annual ryegrass may result from grasses commonly secreting lower concentrations of root exudates and consequently reducing inputs to microorganisms in their rhizosphere leading to reduced enzyme activity (Bridges, 2018). Although winter annual seeding had few impacts, harvest frequency treatments included in this study tended to influence many soil properties. This relationship between harvest frequency and soil health provides essential knowledge because to achieve the benefits of a warm-season perennial pastures it is essential to soil chemical and biological properties to include correct management practices (Yang et al., 2019).

Forage harvest frequency is a key factor for producing sustainable pasturelands as it allows the forage crop to recover and improve or maintain desired species vigor and composition (Kitabe & Tamir, 2005). Additionally, longer harvest intervals maintain quality and quantity of forage for grazing or hay production. In terms of soil health, longer harvest intervals help to improve soil conditions in pasturelands and conserve soil properties over time reducing accelerated soil erosion (Sollenberger et al., 2012). Harvest frequency impacts the rate of plant biomass removal with shorter intervals not allowing the full growth of forage and affecting the top layer of soil where SOM is normally accumulated and has a stronger impact on soil physical properties of the surface soil (Spain et al., 1983; Murphy, 2015). Previous research has recommended waiting at least 4 weeks to harvest the forage pastures (Morrison, 2009). However, in our study some properties like NO_3^--N, NH_4^+-N, SOM, P, and K presented higher concentrations under 8-week harvest frequency than after 4-week harvest intervals. This may suggest that extending the interval between harvests could stimulate greater concentration of important nutrients in the soil. This may increase important properties in soil in preparation for the warm-season pastures.

In this study, forage harvest frequency affected nutrient concentrations, SOM, pH, soil microbial communities, and soil enzymes but changes were site specific. These differences between sites may be caused by soil type, topography, and weather conditions. Hill Farm was the only site presenting significant influence of harvest frequency treatments on relative abundance of microbial communities. In this study, Hill Farm had greater relative abundance of protozoa and AMF under the 4-week harvest interval. In the case of AMF, its ability to survive long periods in soil even under drought conditions makes it a strong microorganism that can withstand disturbances in soil if it has a host plant. This may be a reason why under 4-week harvest frequency, which seems to decrease relative abundance for other microbial communities, AMF increased. Additionally, protozoa's presence could be an explanation for a lack of increases in bacteria under 4-week harvest frequency. It is well known that protozoa feeds on bacteria, potentially controlling bacteria population in soil (Ingham, 1995).

On the other hand, greater relative abundance of saprophytic fungi, GMn, and fungi to bacteria ratio was found under 8-week harvest frequency at Hill Farm. Gram-negative bacteria are very sensitive to soil disturbance (Hoorman, 2012), which may suggest the soil conditions at 8-week harvest frequency may be more supportive compared to 4-week harvest frequency. The increased presence of GMn could be a reason why some nutrients like TC and K were greater in 8-week compared to 4-week harvest frequency in Hill Farm which may be related with the ability and associations of GMn with soil carbon and other nutrients like K (Xue et al., 2018; Meena et al., 2013). According to Meena et al., (2013) there are K solubilizing microorganisms in soil which includes groups of GMn bacteria that play an important role in K cycle in soil, increasing K availability for plant uptake. Total C's increase under 8-week harvest frequency may also be related with the presence of saprophytic fungi that are strongly related with soil mineralization processes and carbon cycling (Jonas et al., 2007).

Mulches create a favorable environment for the development of microorganisms with moderate moisture and temperature (Tu & Toan, 2017; Harris, 1992). Greater relative abundance of actinomycetes, eukaryotes, GMp, and total bacteria was measured under the mulching treatment. The presence of actinomycetes under mulching treatment was also observed in previous studies where mulching has a great impact in actinomycetes in rhizosphere of okra, where the population appeared to increase from sowing to harvest (Muhammed et al., 2015). A possible reason for this could be the higher availability of C in the mulching treatment which stimulates actinomycetes populations in the soil increasing their relative abundance (Pal et al., 2013). Also, GMp bacteria increased in mulching treatment, which could be related to increases in TC and SOM. Other studies presented increased GMp bacteria under mulching films due to SOM mineralization induced by enhancing soil biological activity (Luo et al., 2019). The effects of incorporating mulch include positive impacts on soil physical properties such as maintaining greater soil moisture, moderating soil temperatures, and enhancing SOM (Tu & Toan, 2017; Ni et al., 2016) Additionally, there are reported positive impacts on bacteria, fungi, and actinomycetes population when mulching is included (Muhammed et al., 2015).

Harvest frequency treatments impacted potential NAGase activity at Ben Hur and Brown Loam, resulting in greater enzyme activity under 8-week harvest interval and mulching treatments compared to 4-week harvest intervals. According to Ekenler and M. (2002), mulching as a management practice increased NAGase activity in a no-till soil. A possible explanation for greater NAGase activity in less disturbed soils could be associated with greater soil moisture and SOM in the soil when mulch and no-till practices are incorporated, because of the greater presence of soil microbial biomass stimulating enzymatic activity (Lupwayi et al., 2019; Tabatabai et al., 2010). Potential β-glucosidase was only significantly affected by harvest frequency treatments in all the sites at the Brown Loam site, confirming the statement of Acosta-Martínez et al. (2004), suggesting that NAGase enzyme is a very sensitive enzyme which is rapidly influenced by management practices and for this reason is more likely to find changes in NAGase activity than in β-glucosidase activity.

Significant interactions of forage harvest frequency and cool-season annuals overseeded at the Ben Hur site were observed for Ca, P, and Na in 2020. These three responded differently depending on the cool-season overseeded, but it seems that P increased when overseeding annual ryegrass and harvested at an 8-week frequency. Typically, annual ryegrass needs high to medium soil P to grow, so it is unclear why this increase in soil P was measured under overseeded annual ryegrass compared to other plots (Butler et al., 2007). One possible explanation for increased soil P concentrations, although not related directly to overseeding, may relate to soil pH. Although pH did decrease in 2020 to 6.23 compared to 2017 and 2018, but it still within the range for optimal soil P availability (Adeoye & Agboola, 1985).

There was not a significant impact of cool-season annuals on SOM, however in Ben Hur SOM tended to increase when cool-season annuals were included, suggesting there could be a slow increase of SOM when cool-season annuals are overseeded to a warm-season pasture system (bahiagrass or bermudagrass). The same response to cool-season annuals on SOM happened at Brown Loam and Hill Farm, which further suggested that longer periods of time may be needed to see statistical increases. Overall, the increase of SOM may be related with the sustainable practices included in this study. SOM increases are often stimulated by sustainable practices like mulch incorporation because crop residue serve as a source of organic material and plant nutrients that can be released by mineralization processes (Madgoff & Weil, 2019). Additionally, having diverse species like annual legumes, brassica, and grass increase soil cover which can reduce soil runoff, which may be related with the increase of soil organic carbon which leads to SOM increases in pasturelands (Magdoff et al., 2010).

Nitrate-N and NH_4^+-N decreased in 2020 compared to the 2017 and 2018 samples for all sites, except NH_4^+-N in Hill Farm which slightly increased in 2020. Nitrate-N could be lost via leaching losses or denitrification. The loss of NO_3^--N is common, especially in unfertilized pasture systems (Bridges, 2018). The ammonium-N decrease over time in Ben Hur and Brown Loam may stem from nitrification of NH_4^+ to NO_3^- and a lack of replacement from synthetic fertilizers or legume inputs. Continued plant uptake by perennial grasses and winter-annuals would also contribute for losses of inorganic N (Bowles et al., 2014).

Forage yield results presented interactions of year and harvest frequency in Brown Loam and Hill Farm with increases over time for all the harvest frequency treatments. The mulching treatment seemed to have greater impacts at Brown Loam and Hill Farm, which may be related to soil moisture and increased forage yield of perennial grasses when mulching with legumes (Khare et al., 2014). In the case of Hill Farm, it could be related to the greater relative abundance of actinomycetes found in mulching treatments as these microorganisms can promote plant growth improving the nutrient availability in soil (Bhatti et al., 2017). There was also an interaction between time and cool-season annuals overseeding indicating that when legumes were included in Brown Loam and Ben Hur resulting in greater forage yield compared to remaining treatments. These results are similar to results presented by Sleugh et al. (2000) where legumes improved seasonal distribution of forage yield and was more productive at later harvest. However, the strongest variable influencing forage yield in this study was harvest frequency. Similar studies have also reported that increasing harvest frequency to 8-week intervals compared to 4-week intervals increased forage yield and crude protein yield (Farzinmehr et al., 2020; Jung et al., 1988). According to Farzinmehr et al. (2020), the increased forage concentration in later harvest may be related with the better photosynthetic capability activity which increases CO_2 assimilations and leads to increase DM storage in plant.

Finally, for all sites, depth was a factor significantly affecting all measured soil properties. As expected, some soil properties like SOM and biological activity decreased with increasing depth similar to measurements obtained from row crop studies (Sokołowska et al., 2019; Józefowska et al. 2017). In a perennial grass system with extensive rooting systems, it is expected to have a better distribution of nutrients, C, and soil microbial enzymes through the soil layers. However, the nutrient concentrations decreased in bottom depth which may be caused by the increase of SOM in the top 10 cm, which can influence soil enzymatic activity (Johannes et al., 2017) and thus support greater NAGase activity in the top depth. NAGase activity decreased with depth, which may also be associated with the decrease of pH and TC in bottom depth. Organic C usually decreases with depth (Doran et al., 2018), according to Sokołowska et al. (2019), total organic C and TN decreased with soil depth along with soil microbial biomass and physical soil properties like bulk density, soil porosity, and particle density. Stability of soil aggregation tends to be higher in the top layer of soil, which can also be a reason for soil chemical properties to be greater in surface samples (Burri et al., 2009). A possible explanation is because soil aggregate stability is a key indicator of soil structure, which is commonly related with soil nutrient availability, when soil structure is properly managed the soil nutrient availability in a soil can be enhanced (Bronick & Lal, 2005; Marschner & Rengel, 2012).

Chapter 5. Conclusions

In this study, the effects of cool-season annuals could not be differentiated in nutrient concentrations, microbial relative and absolute abundance, or in potential β-glucosidase activity from the control. However, the effects of cool-season annuals underwent changes in forage yield of warm-season perennial bahiagrass, bermudagrass and in potential β-glucosaminidase activity which may suggest a positive contribution to the soil health of these pastures. On the other hand, the forage harvest frequency seems to affect several properties of soil health in warm-season perennial bermudagrass and bahiagrass. The impact varied in some places possibly due to the unique soil type and topography in each site. Overall, the eight-week harvest frequency and mulching seemed to have had a greater influence on soil health properties and forage yield than the four-week harvest frequency. This may imply the benefit of increasing the recommended four-week harvesting to an eight-week harvesting to enhance soil health in the warm-season perennial pastures distributed in the Southeastern United States.

References

Acosta-Martínez, V., Zobeck, T. M., & Allen, V. (2004). Soil Microbial, Chemical and Physical Properties in Continuous Cotton and Integrated Crop-Livestock Systems. *Soil Science Society of America Journal*, *68*(6), 1875–1884. https://doi.org/10.2136/sssaj2004.1875

Adeoye, G. O., & Agboola, A. A. (1985). Critical levels for soil pH, available P, K, Zn and Mn and maize ear-leaf content of P, Cu and Mn in sedimentary soils of South-Western Nigeria. *Fertilizer Research*, *6*(1), 65–71. https://doi.org/10.1007/bf01058165

Alemu, A., Kröbel, R., McConkey, B., & Iwaasa, A. (2019). Effect of Increasing Species Diversity and Grazing Management on Pasture Productivity, Animal Performance, and Soil Carbon Sequestration of Re-Established Pasture in Canadian Prairie. *Animals*, *9*(4), 127. https://doi.org/10.3390/ani9040127

Alkorta, I., Aizprua, A., Riga, P., Albizu, I., Amezaga, I., & Garbisu, C. (2003). Soil enzyme activities as biological indicators of soil health. *Reviews on Environmental Health*, *18*(1), 65–73.

Almeida, R., Nave, E., & Pinheiro da Mota, R. (2015). Soil quality: Enzymatic activity of soil β-glucosidase. *Global Science Research Journals*, *3*(2), 146–150.

Amorim, H. C., Ashworth, A. J., Moore, P. A., Wienhold, B. J., Savin, M. C., Owens, P. R., Jagadamma, S., Carvalho, T. S., & Xu, S. (2020). Soil quality indices following long-term conservation pasture management practices. *Agriculture, Ecosystems & Environment*, *301*, 107060. https://doi.org/10.1016/j.agee.2020.107060

Beck, P., Gunter, S., & phillips, J. (2006). Evaluation of Supplementation Programs for Growing Cattle Grazing Tall Fescue1. *The Professional Animal Scientist*, *22*(4), 325–333. https://doi.org/10.15232/s1080-7446(15)31115-3

Bhatti, A. A., Haq, S., & Bhat, R. A. (2017). Actinomycetes benefaction role in soil and plant health. *Microbial Pathogenesis*, *111*, 458–467. https://doi.org/10.1016/j.micpath.2017.09.036

Bhowmik, A., Cloutier, M., Ball, E., & Ann Bruns, M. (2017). Underexplored microbial metabolisms for enhanced nutrient recycling in agricultural soils. *AIMS Microbiology*, *3*(4), 826–845. https://doi.org/10.3934/microbiol.2017.4.826

Biederbeck, V., Zentner, R., & Campbell, C. (2005). Soil microbial populations and activities as influenced by legume green fallow in a semiarid climate. *Soil Biology and Biochemistry*, *37*(10), 1775–1784. https://doi.org/10.1016/j.soilbio.2005.02.011

Bottomley, J. S. A. P. S., Weaver, R. W., & Angle, J. S. (1994). *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. Soil Science Society of America.

Bowles, T. M., Acosta-Martínez, V., Calderón, F., & Jackson, L. E. (2014). Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. *Soil Biology and Biochemistry*, *68*, 252–262. https://doi.org/10.1016/j.soilbio.2013.10.004

Bridges, K. M. (2018, October). *Analysis of the soil health of pastures over-seeded with winter annuals.* Louisiana State University. https://digitalcommons-lsu-edu.libezp.lib.lsu.edu/cgi/viewcontent.cgi?article=5786&context=gradschool_dissertations

Briske, D., Derner, J., Brown, J., Fuhlendorf, S., Teague, W., Havstad, K., Gillen, R., Ash, A., & Willms, W. (2008). Rotational Grazing on Rangelands: Reconciliation of Perception and Experimental Evidence. *Rangeland Ecology & Management*, *61*(1), 3–17. https://doi.org/10.2111/06-159r.1

Bronick, C., & Lal, R. (2005). Soil structure and management: a review. *Geoderma*, *124*(1–2), 3–22. https://doi.org/10.1016/j.geoderma.2004.03.005

Brown Loam Exp. Stn. (2021). Delta Agricultural Weather Center. http://deltaweather.extension.msstate.edu/brown-loam-exp-stn

Brummer, E. C., & Moore, K. J. (2000). Persistence of Perennial Cool-Season Grass and Legume Cultivars under Continuous Grazing by Beef Cattle. *Agronomy Journal*, *92*(3), 466–471. https://doi.org/10.2134/agronj2000.923466x

Burri, K., Graf, F., & Böll, A. (2009). Revegetation measures improve soil aggregate stability: a case study of a landslide area in Central Switzerland. *Snow Landsc. Res*, *82*(1), 45–60.

Butler, T. J., Muir, J. P., & Provin, T. L. (2007). Phosphorus Fertilization of Annual Ryegrass and Comparison of Soil Phosphorus Extractants. *Journal of Plant Nutrition*, *30*(1), 9–20. https://doi.org/10.1080/01904160601054825

Byrd, J. D., & Layton, B. (2018). *Forages*. Mississippi State University Extension Service. http://extension.msstate.edu/agriculture/forages

Cano, A., Núñez, A., Acosta-Martinez, V., Schipanski, M., Ghimire, R., Rice, C., & West, C. (2018). Current knowledge and future research directions to link soil health and water conservation in the Ogallala Aquifer region. *Geoderma*, *328*, 109–118. https://doi.org/10.1016/j.geoderma.2018.04.027

Caradus, J. R., & Harris, S. L. (1996). Increased clover content for increased milk production. *Proceedings 48th Ruakura Farmers' Conference*, *48*, 42–49.

Chen, G., & Weil, R. R. (2009). Penetration of cover crop roots through compacted soils. *Plant and Soil*, *331*(1–2), 31–43. https://doi.org/10.1007/s11104-009-0223-7

Cheng, F., Wu, J., & Wang, X. (2014). Genome triplication drove the diversification of Brassica plants. *Horticulture Research, 1*(1). https://doi.org/10.1038/hortres.2014.24

Climate United States - Monthly Averages. (2021). US Climate Data. https://www.usclimatedata.com/

Consumption of mycorrhizal and saprophytic fungi by Collembola in grassland soils. (2007, October 1). ScienceDirect. https://linkinghub.elsevier.com/retrieve/pii/S0038071707002106

Cool-season or Warm-season Grasses. (2018, July 25). Forage Information System. https://forages.oregonstate.edu/regrowth/how-does-grass-grow/grass-types/cool-season-or-warm-season-grasses

Crini, G., & Lichtfouse, E. (2018). *Sustainable Agriculture Reviews 42: Hemp Production and Applications*. Springer.

Dabney, S. M., Delgado, J. A., & Reeves, D. W. (2001). USING WINTER COVER CROPS TO IMPROVE SOIL AND WATER QUALITY. *Communications in Soil Science and Plant Analysis, 32*(7–8), 1221–1250. https://doi.org/10.1081/css-100104110

Dhakal, H., Yang, E. J., Lee, S., Kim, M. J., Baek, M. C., Lee, B., Park, P. H., Kwon, T. K., Khang, D., Song, K. S., & Kim, S. H. (2019). Avenanthramide C from germinated oats exhibits anti-allergic inflammatory effects in mast cells. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-43412-2

Donaghy, P., Bray, S., Gowen, R., Rolfe, J., Stephens, M., Hoffmann, M., & Stunzer, A. (2010). The Bioeconomic Potential for Agroforestry in Australia’s Northern Grazing Systems. *Small-Scale Forestry*, *9*(4), 463–484. https://doi.org/10.1007/s11842-010-9126-y

Doran, J. W., Jones, A. J., Arshad, M., & Gilley, J. (2018). Determinants of Soil Quality and Health. *Soil Quality and Soil Erosion*, 17–36. https://doi.org/10.1201/9780203739266-2

Duncan, E. W., Osmond, D. L., Shober, A. L., Starr, L., Tomlinson, P., Kovar, J. L., Moorman, T. B., Peterson, H. M., Fiorellino, N. M., & Reid, K. (2019). Phosphorus and Soil Health Management Practices. *Agricultural & Environmental Letters*, *4*(1), 190014. https://doi.org/10.2134/ael2019.04.0014

Ekenler, M., & M., T. (2002). β-Glucosaminidase activity of soils: effect of cropping systems and its relationship to nitrogen mineralization. *Biology and Fertility of Soils*, *36*(5), 367–376. https://doi.org/10.1007/s00374-002-0541-x

Ekenler, M., & Tabatabai, M. (2003). Tillage and residue management effects on β-glucosaminidase activity in soils. *Soil Biology and Biochemistry*, *35*(6), 871–874. https://doi.org/10.1016/s0038-0717(03)00094-4

Evers, G. W. (2005). *A Guide to Overseeding Warm-Season Perennial Grasses with Cool-Season Annuals* (Vol. 3). Forage & Grazinglands. https://doi.org/10.1094/FG-2005-0614-01-MG

Evers, G. W. (2011). *A Guide to Overseeding Warm-Season Perennial Grasses with Cool-Season Annuals*. Agricultural Research and Extension Center Overton. http://liveoak.agrilife.org/files/2011/07/Overseeding_Warm_season_PerenialGrasses_14.pdf

Fanin, N., Kardol, P., Farrell, M., Nilsson, M. C., Gundale, M. J., & Wardle, D. A. (2019). The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. *Soil Biology and Biochemistry*, *128*, 111–114. https://doi.org/10.1016/j.soilbio.2018.10.010

Farzinmehr, S., Rezaei, J., & Fazaeli, H. (2020). Effect of harvesting frequency and maturity stage of Jerusalem artichoke forage on yield, chemical composition and in vitro fermentation of the tubers and forage. *Spanish Journal of Agricultural Research*, *18*(2), e0602. https://doi.org/10.5424/sjar/2020182-15379

Gates, R. N., Quarin, C. L., & Pedreira, C. G. (2016). Bahiagrass. *Agronomy Monographs*, 651–680. https://doi.org/10.2134/agronmonogr45.c19

Gianfreda, L., & Ruggiero, P. (2006). Enzyme Activities in Soil. *Soil Biology*, 257–311. https://doi.org/10.1007/3-540-29449-x_12

Haby, V. A., Stewart, W. M., & Leonard, A. T. (2008). TIFTON 85 BERMUDAGRASS RESPONSE TO POTASSIUM, CHLORIDE AND SULFUR AT TWO NITROGEN RATES. *Great Plains Soil Fertility Conference.*, *12*, 134–139.

Han, K. J., Alison, M. W., Pitman, W. D., & McCormick, M. E. (2012). Contributions of Overseeded Clovers to Bermudagrass Pastures in Several Environments. *Crop Science*, *52*(1), 431–441. https://doi.org/10.2135/cropsci2011.08.0401

Hancock, D. W. (2006, June 1). *Bermudagrass in Georgia*. University of Georgia Extension. https://extension.uga.edu/publications/detail.html?number=B1362&title=The%20Management%20and%20Use%20of%20Bahiagrass

Hancock, D., Waltz, F. C., Tucker, J. J., Anderson, B., & Hudson, W. G. (2010, March 1). *Turfgrass Pest Control Recommendations for Professionals*. University of Georgia Extension. https://extension.uga.edu/publications/detail.html?number=B1484&title=Managing%20Bermudagrass%20Stem%20Maggots

Haramoto, E. R., & Gallandt, E. R. (2005). Brassica cover cropping: I. Effects on weed and crop establishment. *Weed Science*, *53*(5), 695–701. https://doi.org/10.1614/ws-04-162r.1

Hood-Nowotny, R., Umana, N. H. N., Inselbacher, E., Oswald- Lachouani, P., & Wanek, W. (2010). Alternative Methods for Measuring Inorganic, Organic, and Total Dissolved Nitrogen in Soil. *Soil Science Society of America Journal*, *74*(3), 1018–1027. https://doi.org/10.2136/sssaj2009.0389

Hoorman, J. J. (2016). *Role of Soil Bacteria: Update and Revision*. https://mccc.msu.edu/wp-content/uploads/2016/10/OH_2016_Role-of-soil-bacteria_Update-and-revision-May12_2016.pdf

Idowu, J., Ghimire, R., Flynn, R., & Ganguli, A. (2019). *NMSU: Soil Health—Importance, Assessment, and Management*. New Mexico State University. https://aces.nmsu.edu/pubs/_circulars/CR694B/welcome.html

Iglesias, & Lloveras. (1998). Annual cool-season legumes for forage production in mild winter areas. *Grass and Forage Science*, *53*(4), 318–325. https://doi.org/10.1046/j.1365-2494.1998.00135.x

Iii, R. T. L. (2000). *Soil Microbiology, 2nd Edition* (2nd ed.). Wiley.

Jani, A. D., Grossman, J. M., Smyth, T. J., & Hu, S. (2015). Influence of soil inorganic nitrogen and root diameter size on legume cover crop root decomposition and nitrogen release. *Plant and Soil*, *393*(1–2), 57–68. https://doi.org/10.1007/s11104-015-2473-x

Jastrow, J. D., & Miller, R. M. (1997). *Soil Aggregate Stabilization and Carbon Sequestration: Feedbacks through Organomineral Associations* (1st ed.). CRC Press.

Johannes, A., Matter, A., Schulin, R., Weisskopf, P., Baveye, P. C., & Boivin, P. (2017). Optimal organic carbon values for soil structure quality of arable soils. Does clay content matter? *Geoderma*, *302*, 14–21. https://doi.org/10.1016/j.geoderma.2017.04.021

Johns, C. (2017). Living Soils: The Role of Microorganisms in Soil Health. *Australian Soils and Landscape, An Illustrated Compendium*. Published.

Józefowska, A., Pietrzykowski, M., Woś, B., Cajthaml, T., & Frouz, J. (2017). The effects of tree species and substrate on carbon sequestration and chemical and biological properties in reforested post-mining soils. *Geoderma*, *292*, 9–16. https://doi.org/10.1016/j.geoderma.2017.01.008

Kelly, C., Schipanski, M. E., Tucker, A., Trujillo, W., Holman, J. D., Obour, A. K., Johnson, S., Brummer, J. E., Haag, L., & Fonte, S. J. (2021). Dryland cover crop soil health benefits are maintained with grazing in the U.S. High and Central Plains. *Agriculture, Ecosystems & Environment*, *313*, 107358. https://doi.org/10.1016/j.agee.2021.107358

Khare, D., Patra, D., Mondal, A., & Kundu, S. (2014). Impact of landuse/land cover change on run-off in a catchment of Narmada river in India. *Applied Geomatics*, *7*(1), 23–35. https://doi.org/10.1007/s12518-014-0148-6

Knudsen, G. R., Stack, J. P., Schuhmann, S. O., Orr, K., & LaPaglia, C. (2006). Individual-Based Approach to Modeling Hyphal Growth of a Biocontrol Fungus in Soil. *Phytopathology®*, *96*(10), 1108–1115. https://doi.org/10.1094/phyto-96-1108

Kohler, T. J., Peter, H., Fodelianakis, S., Pramateftaki, P., Styllas, M., Tolosano, M., de Staercke, V., Schön, M., Busi, S. B., Wilmes, P., Washburne, A., & Battin, T. J. (2020). Patterns and Drivers of Extracellular Enzyme Activity in New Zealand Glacier-Fed Streams. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.591465

Kramberger, B., Gselman, A., Kristl, J., Lešnik, M., ŠUštar, V., Muršec, M., & Podvršnik, M. (2014). Winter cover crop: the effects of grass–clover mixture proportion and biomass management on maize and the apparent residual N in the soil. *European Journal of Agronomy, 55*, 63–71. https://doi.org/10.1016/j.eja.2014.01.001

Leanne Dillard, S., Hancock, D. W., Harmon, D. D., Kimberly Mullenix, M., Beck, P. A., & Soder, K. J. (2018). Animal performance and environmental efficiency of cool- and warm-season annual grazing systems. *Journal of Animal Science*, *96*(8), 3491–3502. https://doi.org/10.1093/jas/sky025

Lee, K., & Pankhurst, C. (1992). Soil organisms and sustainable productivity. *Soil Research, 30*(6), 855. https://doi.org/10.1071/sr9920855

Lemus, R. (2018, September). *Forage: Bermudagrass*. Mississippi State University Extension Service. http://extension.msstate.edu/publications/forage-bermudagrass

Liang, S., Grossman, J., & Shi, W. (2014). Soil microbial responses to winter legume cover crop management during organic transition. *European Journal of Soil Biology*, *65*, 15–22. https://doi.org/10.1016/j.ejsobi.2014.08.007

Lithourgidis, A., Vasilakoglou, I., Dhima, K., Dordas, C., & Yiakoulaki, M. (2006). Forage yield and quality of common vetch mixtures with oat and triticale in two seeding ratios. *Field Crops Research*, *99*(2–3), 106–113. https://doi.org/10.1016/j.fcr.2006.03.008

Local Weather Forecast, News and Conditions | Weather Underground. (2021). Weather Underground. https://www.wunderground.com/

Lupwayi, N. Z., Zhang, Y., Hao, X., Thomas, B. W., Eastman, A. H., & Schwinghamer, T. D. (2019). Linking soil microbial biomass and enzyme activities to long-term manure applications and their nonlinear legacy. *Pedobiologia*, *74*, 34–42. https://doi.org/10.1016/j.pedobi.2019.04.001

Magdoff, F., Es, H., & Mason, J. (2010). *Building Soils for Better Crops* (3rd ed.). SARE Outreach.

Magdoff, F., & Weil, R. R. (2019). *Soil Organic Matter in Sustainable Agriculture (Advances in Agroecology)* (1st ed.). CRC Press.

Marschner, P., & Rengel, Z. (2012). Nutrient Availability in Soils. *Marschner's Mineral Nutrition of Higher Plants*, 315–330. https://doi.org/10.1016/b978-0-12-384905-2.00012-1

McKenna, P., Cannon, N., Conway, J., Dooley, J., & Davies, W. P. (2018). Red clover (Trifolium pratense) in conservation agriculture: a compelling case for increased adoption. *International Journal of Agricultural Sustainability*, *16*(4–5), 342–366. https://doi.org/10.1080/14735903.2018.1498442

Meena, V. S., Maurya, B., & Verma, J. P. (2014). Does a rhizospheric microorganism enhance K+ availability in agricultural soils? *Microbiological Research*, *169*(5–6), 337–347. https://doi.org/10.1016/j.micres.2013.09.003

Meter, K., & Goldenberg, M. P. (2014). *Overview of Mississippi Farm & Food Economy*. Crossroads Resource Center.

Natural Resources Conservation Service. (2016). *Grazing Management and Soil Health*. United States Department of Agriculture. https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcseprd621806.pdf

Natural Resources Conservation Service. (2019). *Web Soil Survey*. United States Department of Agriculture. https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx

N'Dayegamiye, A., Whalen, J. K., Tremblay, G., Nyiraneza, J., Grenier, M., Drapeau, A., & Bipfubusa, M. (2015). The Benefits of Legume Crops on Corn and Wheat Yield, Nitrogen Nutrition, and Soil Properties Improvement. *Agronomy Journal*, *107*(5), 1653–1665. https://doi.org/10.2134/agronj14.0416

Nelson, D. W., & Sommers, L. E. (1996). Total Carbon, Organic Carbon, and Organic Matter. *SSSA Book Series*, 961–1010. https://doi.org/10.2136/sssabookser5.3.c34

Nickerson, C., Ebel, R., Borchers, A., & Carriazo, F. (2012). *Major Uses of Land in the United States, 2007*. CreateSpace Independent Publishing Platform.

Pankhurst, C. E., Ophel-Keller, K., Doube, B. M., & Gupta, V. V. S. R. (1996). Biodiversity of soil microbial communities in agricultural systems. *Biodiversity and Conservation*, *5*(2), 197–209. https://doi.org/10.1007/bf00055830

Parham, J., & Deng, S. (2000). Detection, quantification and characterization of β-glucosaminidase activity in soil. *Soil Biology and Biochemistry*, *32*(8–9), 1183–1190. https://doi.org/10.1016/s0038-0717(00)00034-1

Passioura, J. B. (1991). An Impasse in Plant Water Relations? *Botanica Acta*, *104*(6), 405–411. https://doi.org/10.1111/j.1438-8677.1991.tb00250.x

Pasture Resources | NRCS. (2019). United States Department of Agriculture. https://www.nrcs.usda.gov/wps/portal/nrcs/main/national/landuse/rangepasture/pasture/

Paul, E. (2014). *Soil Microbiology, Ecology and Biochemistry* (4th ed.). Academic Press.

Procedures. (2015, November 23). LSU AgCenter. https://www.lsuagcenter.com/portals/our_offices/departments/spess/servicelabs/soil_testing_lab/procedures

Range and Pastureland Overview | NRCS. (2017). United States Department of Agriculture. https://www.nrcs.usda.gov/wps/portal/nrcs/detail/national/landuse/rangepasture/?cid=nrcsdev11_001074

Reeve, J., Hoagland, L., Villalba, J., Carr, P., Atucha, A., Cambardella, C., Davis, D., & Delate, K. (2016). Organic Farming, Soil Health, and Food Quality: Considering Possible Links. *Advances in Agronomy*, 319–367. https://doi.org/10.1016/bs.agron.2015.12.003

Rutkowska, B., Szulc, W., Bomze, K., Gozdowski, D., & Spychaj-Fabisiak, E. (2014). Soil factors affecting solubility and mobility of zinc in contaminated soils. *International Journal of Environmental Science and Technology*, *12*(5), 1687–1694. https://doi.org/10.1007/s13762-014-0546-7

Sanderson, M. A., Skinner, R. H., Barker, D. J., Edwards, G. R., Tracy, B. F., & Wedin, D. A. (2004). Plant Species Diversity and Management of Temperate Forage and Grazing Land Ecosystems. *Crop Science*, *44*(4), 1132–1144. https://doi.org/10.2135/cropsci2004.1132

Sanderson, M. A., Soder, K. J., Muller, L. D., Klement, K. D., Skinner, R. H., & Goslee, S. C. (2005). Forage Mixture Productivity and Botanical Composition in Pastures Grazed by Dairy Cattle. *Agronomy Journal*, *97*(5), 1465–1471. https://doi.org/10.2134/agronj2005.0032

Scheu, S., Schlitt, N., Tiunov, A. V., Newington, J. E., & Jones, H. T. (2002). Effects of the presence and community composition of earthworms on microbial community functioning. *Oecologia*, *133*(2), 254–260. https://doi.org/10.1007/s00442-002-1023-4

Schoenholtz, S. H. (2000). *A Review of Chemical and Physical Properties as Indicators of Forest Soil Quality: Challenges and Opportunities*. DigitalCommons@USU. https://digitalcommons.usu.edu/wats_facpub/14/

Schutter, M. E., & Dick, R. P. (2000). Comparison of Fatty Acid Methyl Ester (FAME) Methods for Characterizing Microbial Communities. *Soil Science Society of America Journal*, *64*(5), 1659–1668. https://doi.org/10.2136/sssaj2000.6451659x

Selvasekaran, P., & Chidambaram, R. (2020). Agriculturally Important Fungi for Crop Protection. *Fungal Biology*, 1–53. https://doi.org/10.1007/978-3-030-48474-3_1

Silveira, M. L., Haby, V. A., & Leonard, A. T. (2007). Response of Coastal Bermudagrass Yield and Nutrient Uptake Efficiency to Nitrogen Sources. *Agronomy Journal*, *99*(3), 707–714. https://doi.org/10.2134/agronj2006.0200

Simard, S. W., Roach, W. J., Defrenne, C. E., Pickles, B. J., Snyder, E. N., Robinson, A., & Lavkulich, L. M. (2020). Harvest Intensity Effects on Carbon Stocks and Biodiversity Are Dependent on Regional Climate in Douglas-Fir Forests of British Columbia. *Frontiers in Forests and Global Change*, *3*. https://doi.org/10.3389/ffgc.2020.00088

Sleugh, B., Moore, K. J., George, J. R., & Brummer, E. C. (2000). Binary Legume-Grass Mixtures Improve Forage Yield, Quality, and Seasonal Distribution. *Agronomy Journal*, *92*(1), 24–29. https://doi.org/10.2134/agronj2000.92124x

Soil Health | NRCS Soils. (2020). United States Department of Agriculture. https://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/health/

Sokołowska, J., Józefowska, A., Woźnica, K., & Zaleski, T. (2019). Interrelationship between soil depth and soil properties of Pieniny National Park forest (Poland). *Journal of Mountain Science*, *16*(7), 1534–1545. https://doi.org/10.1007/s11629-019-5441-8

Song, Y., Jiang, L., Song, C., Wang, X., Ma, X., Zhang, H., Tan, W., Gao, J., & Hou, A. (2021). Microbial abundance and enzymatic activity from tussock and shrub soil in permafrost peatland after 6-year warming. *Ecological Indicators*, *126*, 107589. https://doi.org/10.1016/j.ecolind.2021.107589

St. Luce, M., Ziadi, N., Zebarth, B. J., Grant, C. A., Tremblay, G. F., & Gregorich, E. G. (2014). Rapid determination of soil organic matter quality indicators using visible near infrared reflectance spectroscopy. *Geoderma*, *232–234*, 449–458. https://doi.org/10.1016/j.geoderma.2014.05.023

Tabatabai, M. A., Ekenler, M., & Senwo, Z. N. (2010). Significance of Enzyme Activities in Soil Nitrogen Mineralization. *Communications in Soil Science and Plant Analysis*, *41*(5), 595–605. https://doi.org/10.1080/00103620903531177

Tu, V. N., & Toan, N. V. (2017). Effects of Microbial Organic Fertilizer and Mulch to Population and Bioactivity of Beneficial Microorganisms in Tea Soil in Phu Tho, Viet Nam. *International Journal of Agricultural Technology*, *13*(4), 469–484.

Tulley, W. (2015). Growing, harvesting and feeding grass part 1: principles of pasture management. *Livestock*, *20*(4), 212–215. https://doi.org/10.12968/live.2015.20.4.212

Turco, R. F., Kennedy, A. C., & Jawson, M. D. (2015). Microbial Indicators of Soil Quality. *SSSA Special Publications*, 73–90. https://doi.org/10.2136/sssaspecpub35.c5

Twidwell, E. (2000, June 12). *Performance of Russell Bermudagrass*. LSU AgCenter. https://agrilife.org/spfcic/files/2013/02/twidwell.pdf

Villamil, M. B., Bollero, G. A., Darmody, R. G., Simmons, F. W., & Bullock, D. G. (2006). No-Till Corn/Soybean Systems Including Winter Cover Crops. *Soil Science Society of America Journal*, *70*(6), 1936–1944. https://doi.org/10.2136/sssaj2005.0350

Voroney, R. (2007). The Soil Habitat. *Soil Microbiology, Ecology and Biochemistry*, 25–49. https://doi.org/10.1016/b978-0-08-047514-1.50006-8

Watanabe, M., Netzer, F., Tohge, T., Orf, I., Brotman, Y., Dubbert, D., Fernie, A. R., Rennenberg, H., Hoefgen, R., & Herschbach, C. (2018). Metabolome and Lipidome Profiles of Populus × canescens Twig Tissues During Annual Growth Show Phospholipid-Linked Storage and Mobilization of C, N, and S. *Frontiers in Plant Science, 9*. https://doi.org/10.3389/fpls.2018.01292

Weil, R., White, C., & Lawley, Y. (2009, January). *Forage Radish: New Multi-Purpose Cover Crop for the Mid Atlantic*. University of Maryland. http://northeastcovercrops.com/wp-content/uploads/2018/03/Forage-Radish-New-Multi-Purpose-Cover-Crop-for-the-Mid-Atlantic.pdf

Went, F. W., & Stark, N. (1968). THE BIOLOGICAL AND MECHANICAL ROLE OF SOIL FUNGI. *Proceedings of the National Academy of Sciences*, *60*(2), 497–504. https://doi.org/10.1073/pnas.60.2.497

Wick, A., Berti, M., Lawley, Y., & Liebig, M. (2017). *Integration of annual and perennial cover crops for improving soil health*. Soil Health and Intensification of Agroecosystems.

Williams, S. M., & Weil, R. R. (2004). Crop Cover Root Channels May Alleviate Soil Compaction Effects on Soybean Crop. *Soil Science Society of America Journal*, *68*(4), 1403–1409. https://doi.org/10.2136/sssaj2004.1403

Zhang, Y., Griffin, A., & Edwards, M. (2008). Nitrification in Premise Plumbing: Role of Phosphate, pH and Pipe Corrosion. *Environmental Science & Technology*, *42*(12), 4280–4284. https://doi.org/10.1021/es702483d

Vita

Carolina Muela, born in Quito, Ecuador received her bachelor's degree on Environmental Sciences from Escuela Agricola Panamerica Zamorano at Honduras. She then started an internship in the Department of Plant Pathology at Louisiana State University. She was accepted into the master's graduate program in SPESS at Louisiana State University and started in 2019 under the direction of Dr. Lisa M. Fultz. She is planning to receive her master's diploma this August 2021.